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Contributors are requested to be clear and concise. Manuscripts should not exceed 8,000 words and should be in a final form for the press. Each paper should start with a short summary which should be an abstract of the whole paper, complete and clear in itself, and not over 3 per cent. of the length of the paper. The introduction and reviews of literature should be restricted to closely pertinent papers.

The manuscript should be typewritten on one side of the paper only, with wide margins and be double spaced throughout including titles, footnotes, literature citations and legends. Symbols, formulae and equations must be written clearly and with great care. Scientific names of genera and species are printed in italics and should be underlined in the typescript. Too many tables, graphs, etc. should be avoided. Each table should be typed on a separate sheet with its proper position marked in the text in pencil.

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Kinetics of Some Reactions in Solution

BY

S. V. ANANTAKRISHNAN

Introductory Note

The following pages formed the subject of Five lectures delivered in the different colleges doing post graduate work in Madras under the Sir Subramania Iyer Endowment of the University of Madras. They form a review of the work that has been carried out during the last twenty years in Madras Christian College, Tambaram under difficult conditions, administrative and financial. I must acknowledge my indebtedness to the enthusiasm and hard work of a number of my colleagues and students whose names figure in the references at the end of each talk. I should also thank the University of Madras for having given me this opportunity of reviewing our work and placing it before a wider audience.

LECTURE I

GENERAL INTRODUCTION

Chemical Kinetics is concerned with the speed and mechanism of chemical reactions. Several factors have to be taken into account in using the measurements of speed of a reaction in order to elucidate the mechanism of a reaction. The earliest observation in this direction was that of Wilhelmy[1] on the inversion of cane sugar while the earliest fundamental law was enunciated by Guldberg and Waage[2] as the law of Mass Action. The influence of temperature and an explanation of the temperature coefficient was clearly put forward by Arrhenius.[3]

The basic idea of Arrhenius is the postulate that as a result of collision, some molecules get activated, the energy of activation being defined by the rate equation

$$k = Se^{-E/RT}$$

It is implicit that this activation energy is independent of temperature and only the activated molecules undergo the transformation,

TABLE I

Reaction Order	Differential Equation	Integrated Form	Time for Half Change
0	$\frac{dx}{dt}=k$	$k=\frac{a_o-a}{t}=\frac{x}{t}$	$\frac{a}{2k}$
1	$\frac{dx}{dt}=k(a-x)$	$k=\frac{1}{t}ln\frac{a}{a-x}$	$\frac{1}{k}ln\ 2$
2	$\frac{dx}{dt}=k(a-x)\ (b-x)$	$k=\frac{1}{t(a-b)}ln\frac{b(a-x)}{a(b-x)}$	$\left(\frac{1}{ka}\right)$ with reactants at same concentration
3	$\frac{dx}{dt}=k(a-x)\ (b-x)\ (c-x)$	$\left(k=\frac{1}{t}\frac{1}{(a-b)(a-c)}ln\frac{a}{a-x}\right)+\ldots\ldots\ldots$	$\left(\frac{3}{2ka}\right)$ all reactants at same concentration

The rate constant in the equation is a quantity usually calculated from experimental data on the variations of concentrations of reactants with time. These are conventionally derived using appropriate differential equations and their integrated forms. Making arbitrary assumptions of reaction orders the expressions in Table I can be derived.

If the reaction is simple and there are no complications, the rate equation can be used to evaluate the two parameters by measuring the temperature coefficient of the reaction rate. The normal run of reactions, however, rarely follows this ideal behaviour. The deviations are clearly indicated by abnormal values of the pre-exponential factor of the rate equation, the reaction rate not conforming to any simple order, the rate constent showing no linear relationship with the reciprocal of temperature, etc.[4, 5] In using these, it is also implicit that the course of the reaction is homogeneous.

It is reasonable to expect that the collision theory can be tested if one had a bimolecular reaction free from other complications. The number of such systems have been progressively diminishing and it is no exaggeration to state that there are none at present. However, the combination of hydrogen and iodine as well as the dissociation of hydrogen iodide form the most favourable examples. The thorough study of Bodenstein[6] of this reaction has enabled McC. Lewis[7] to test the Arrhenius equation and a remarkable agreement between calcuated and experimental values has been observed. Sullivan has shown that even this reaction is not free from complications.[8]

One of the difficulties of the collision hypothesis has been the problem of unimolecular reactions. Hinshelwood's generalization[9] is the first significant analysis, based on the assumption that all molecules possessing a minimum energy corresponding to the activation energy have a specific reaction rate independent of the amount of excess energy. The contribution is noteworthy for introducing a term in the rate equation to allow for molecular complexity. A consequence of the picture used is that a plot of $1/k$ against $1/c$ should be a straight line, a generalization which is not strictly obeyed. As the concentration tends to 0, the reaction tends to give a second order constant indicating the formation of active molecules by binary collision as a determining factor.

The Arrhenius theory has as its basis the thermodynamic study of equilibria by van't Hoff, but makes no assumptions as to a possi-

ble intermediate stage between reactants and products. An alternative approach initially indicated by Marcelin[10] and developed by Rice[11] Eyring[12] and Polanyi[13] is generally described as the Transition State method. The method postulates the activation of a molecule A to an excited state A‡ with an energy greater than or equal to the activation energy and the excited state is given a configuration with the maximum potential energy. This constitutes the transition state and lies at the saddle point of the energy surface (Figure 1). The method has been elegantly presented by Wigner.[14]

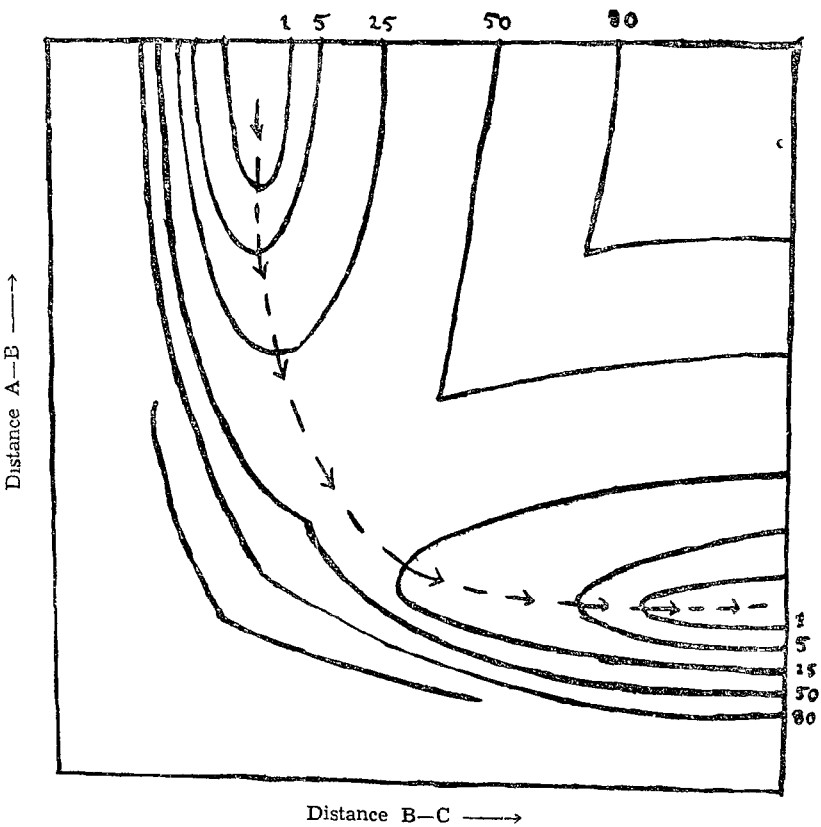


FIG. 1. Saddle point of energy contours in reaction co-ordinate

Three basic assumptions are used. First, the comparatively slow motion of nuclei is followed by rapid motion of electrons so as to be in the lowest quantum state for every position of the nuclei. The motion of the nuclei can be described by classical mechanics

with such quantum corrections as may be necessary. Lastly, all systems crossing a potential barrier are reacting ones. With these assumptions a general thermodynamic theory has been developed involving a hypothetical equilibrium constant $K^{\ddagger}$ between the activated complex of the transition state and the reactants. An important result of this approach is a modification of the rate equation. The pre-exponential function is replaced by two terms, kT/h which has the dimensions of a frequency and $e^{\Delta S/RT}$ introducing the concept of entropy of activation.

The success of the theory of absolute reaction rates, as the method is termed by Eyring and his associates, lies in its ability to evaluate the activation energy of a reaction from fundamental properties of the bonds broken and the bonds formed. This has been illustrated for the reaction between atomic hydrogen and hydrogen molecule by Polanyi[15] and for the ethylene-bromine reaction by Eyring.[16] Though the method in general is applicable to three electron and four electron systems, the very nature of the data required for the calculations preclude any large degree of accuracy. With complex systems even this is not practicable.

While the study of gaseous systems lends itself to reasonable theoretical analysis using concepts of statistical mechanics, a considerable amount of the work on reaction kinetics is with solutions. Several complications are introduced by the presence of the solvent which will be dealt with more fully in the last lecture in this series. An important aspect, however, is the question of collision frequency. Assuming a quasi-crystalline structure for the liquid state Fowler and Slater[17] have shown that the ratio of the number of collisions in solution to that in the liquid state is given by $\frac{3}{4}\pi\ (\sigma^3/a\sigma^3)$. This requires a further correction for repetitive encounters possible in solution but not in the gas phase. Using a lattice model Rabinowitch[18] had calculated the collision frequency by an essentially similar approach except for a difference in the mechanism of diffusion. Solution Kinetics bring out clearly the limitations on the theoretical side whether one adopts the collision or the transition state method.

A significant feature of solution kinetics is the variety of complex behaviour of reacting systems. In gas reactions, bimolecular encounters are the commonest and a certain number of unimolecular processes are known. The only known instances of termolecular reactions are those involving nitrogen monoxide (Nitric oxide). On the other hand, in solutions we have quite a few

instances where one comes across reaction orders higher than two or three and no great significance can be attached to a reaction order except as an indication of the complex nature of the reaction.

Two common complications should be mentioned here. We have very often to deal with concurrent reactions with differing molecularities or differing orders. For a system showing concurrent first and second orders, using the same concentration of reactants, the rate expression leads to the form

$$k_1 = \frac{1}{t} ln \frac{a-\alpha x}{a-x}$$

where α is given by $k_2 a/(k_1+k_2 a)$. This renders the evaluation of rate constants difficult. However, by transforming the differential equation

$$\frac{dx}{dt} = k_1 (a-x) + k_2 (a-x)^2$$

into a linear form, a graphical solution giving values of rate constants of reasonable accuracy can be obtained. A similar approach is possible where concurrent reactions of second and third orders are present. In addition, the unimolecular or bimolecular mechanisms and sometimes both can be modified by the environment of the reacting system, as we shall see later on.

Neither the rate constant nor the Arrhenius parameters by themselves give us any indication of the course of a reaction. They are generally linked up with a tentative reaction mechanism. One has often to consider quite a few alternative mechanisms. Even though our main studies are with reactions in solution, for the sake of simplicity the decomposition of dinitrogen pentoxide will serve as a convenient example of such an approach. Originally shown as an example of a unimolecular reaction both in the gas phase and in solution, the reaction has revealed several variations. Among several mechanisms, only two will be considered here.

1. Busse and F. Daniels[19]

(a) $N_2O_5 \xrightarrow{1} N_2O_3 + O_2$ slow

(b) $N_2O_3 \xrightarrow{2} NO + NO_2$ fast

(c) $NO + N_2O_5 \xrightarrow{3} 3\ NO_2$ fast

2. Ogg[20]

(a) $$N_2O_5 \underset{2}{\overset{1}{\rightleftharpoons}} NO_2 + NO_3$$

(b) $$NO_2 + NO_3 \xrightarrow{3} NO + O_2 + NO_2$$

(c) $$NO + NO_3 \xrightarrow{4} 2\,NO_2$$

$k_2 > k_3$, $k_4 > k_3$.

The first mechanism is a conventional pattern to account for the clear unimolecular decomposition which is different from the stoichiometric picture. The second mechanism indicates the composite nature of the first order rate constant. Where simple molecules of this type are involved, one useful approach is an analysis of the electron systems involved. The rate determining step in the Daniels mechanism is one in which the products violate the spin conservation rules, and may also require a very much lower frequency factor. The mechanism has thus to be rejected. It is a wholesome rule in Reaction Kinetics studies that no mechanism is final. It is difficult to prove a mechanism but it is always on the cards that one is disproved. The example considered just now is a typical instance.

When we turn to complex organic compounds in solution or to complex inorganic compounds the position is quite obscure. If we consider a system capable of prototropic change and subject to acid-base catalysis, two distinct mechanisms are possible. It is to be presumed that the concentrations of acid or base remains constant so that each stage represents a unimolecular process. Following Bell[21] we can represent these as follows:

(I) $$HS{+}{=}\Sigma A_i \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} HSH^+ + \Sigma B_i \xrightarrow{k_2} SH + \Sigma A_i$$

(II) $$B_1 + HS + A_1 \rightleftharpoons A_1 + SH + B_2 .$$

The latter mechanism represents what may be termed a concerted mechanism. It will be possible to distinguish between the two processes only under experimental conditions which preclude ionpair formation.

In postulating reaction mechanisms it is a general practice to represent a slow stage as the rate determining step for the reaction. This is quite often an ideal situation and instances will not be wanting where this is not the case. The reaction will then fail to conform to a simple reaction order and higher orders will be the result.

An aspect of solution reactions which adds to the difficulties of mechanistic studies is the variable degree of solvation of the reactants, transition state and products. The influence of solvent will depend on the extent of such solvation. While we can have some idea of the degree of solvation of the reactants and products, that of the transition state depends on the topology and is at best an intelligent guess. The transition state model gives us a reasonable qualitative picture. Using the general picture

$$A + B + \ldots \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [X] \xrightarrow{k_r} \text{Products}$$

the concentration of the transition state will be given by the equilibrium constant $K^{\ddagger} = k_1/k_{-1}$ and the activity of the reactants. The experimentally observed rate constant will be given by $k_r K^{\ddagger}$ (activity coefficient of reactants). While the activity coefficients of the reactants may be determined experimentally or computed from different theories of solution, the partition functions and activity of the transition state has to be assumed using reasonable models for the reaction and theories of solvation of the transition state. The rate of conversion of this state to the products, k_r, is essentially a vibration frequency pertaining to the bond undergoing transformation and as such may not be influenced by the solvent. The result is that differences in reaction rates are reduced to differences in thermodynamic equilibrium constant.

The existence of some correlation with the characteristic frequency has been noticed in some reactions[22] but as has been indicated there, the linear correlation observed is restricted by the condition that the mechanism should be the same for the series of compounds compared. One might almost say that a deviation from a linear relationship is diagnostic of a mechanistic change.

A further instance of this is provided by the correlation between energies of activation and heats of reaction. If the potential energy curves on the reaction co-ordinate are drawn, we have the

picture indicated (Fig. 2). From this, extending to a series of similar compounds that is possible with organic compounds, one can

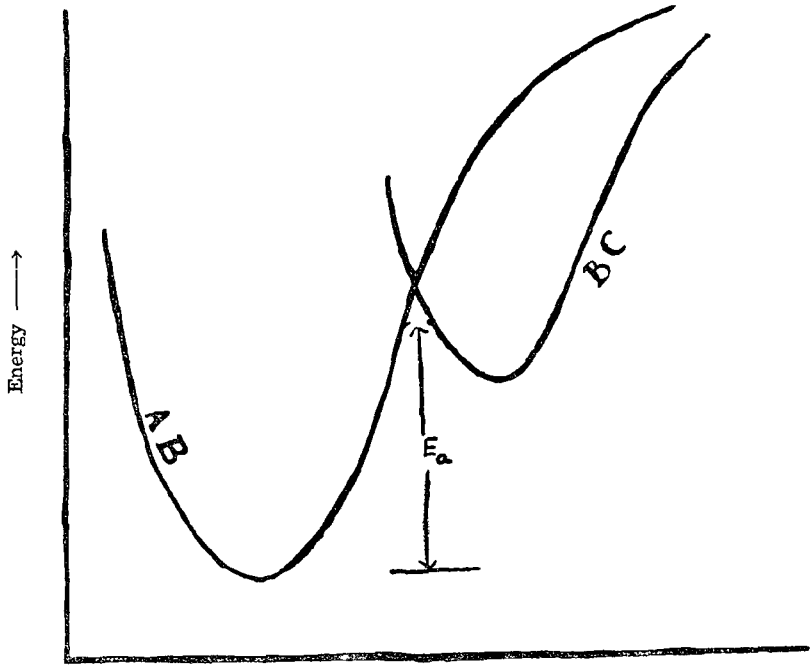


FIG. 2. Potential Energy diagram along reaction path

expect such a correlation. This was first postulated by Evans and Polanyi,[23] in the reaction between sodium atoms and alkyl halides and extended by Butler and Polanyi[24] in their pyrolytic studies. The relationship

$$Q = -\alpha H \qquad 0 < \alpha < 1$$

was found to hold good within the limits of experimental error. A linear relationship is then observable between activation energy and bond energy and, in its turn, with the characteristic frequency used in the Morse equation for potential energy curves. These generalizations have been further strengthened by Russian observations reported by Semenov.[25]

For a study of reaction mechanisms, the kinetic study of reactions of inorganic systems under controlled conditions is a comparatively recent field and ideas developed in the study of reactivity of organic systems have been of considerable help. It will then

be useful to examine these in some detail. The reactions can be broadly divided into substitution and addition reactions. While the latter are essentially four centre systems the former can be either three centre or four centre ones. We can broadly picture them thus:

Substitution: (a) $A + B{-}C \rightarrow A{-}B + C$

(b)
$$\begin{matrix} A & C \\ | & | \\ B & D \end{matrix} \rightarrow \begin{matrix} & A{-}C \\ + & \\ & B{-}D \end{matrix}$$

Addition:
$$\begin{matrix} A & C \\ \| & | \\ B & D \end{matrix} \rightarrow \begin{matrix} A{-}C \\ | \\ B{-}D \end{matrix}$$

The topology of the transition state has then to be decided in relation to the mechanism.

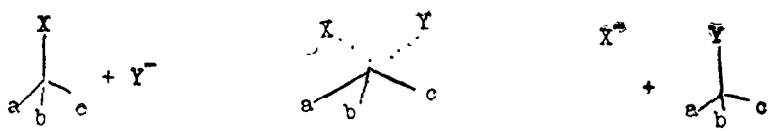
One of the most thorough studies have been on substitution at a saturated carbon atom, by Ingold and his collaborators.[26] These give us the following alternative modes taking any system where substituents are distributed tetrahedrally. A similar picture has been indicated by Polanyi[27] in his gas reaction studies as well.

I Initial State $A \quad B{-}C$

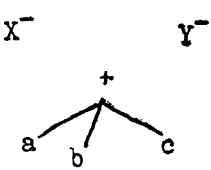
Transition State $A....B....C$

Final State $A{-}B \quad C$

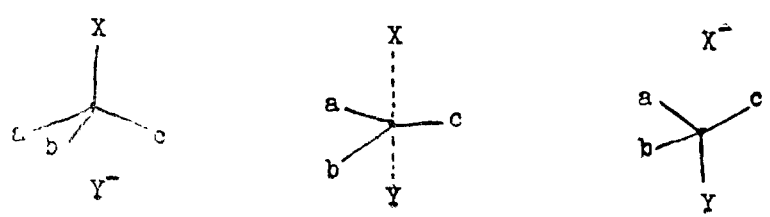
Initial State Transition State Final State



II



or



Where the formation of a cation is slow and constitutes the rate determining step, first order rate constants may be expected. If this is not the case, a second order rate will be the result. In the gaseous state, formation of a charged body will be unlikely in the course of a reaction and free radical mechanisms will prevail. Again the kinetics will depend on the rate determining step.

"The advent of radiation chemistry, and electric discharges as well as gas phase processes in flames indicated no doubt the possibility of such ion-molecule reactions, but it is only the development of mass spectrometer techniques that have brought out the importance of these."[27a].

We have noticed earlier how an important contribution to the activation energy is made by the bond dissociation energy. The wide range of this quantity for apparently the same bond is clearly seen from Table II (Data from Semenov[28]). The distinct changes

TABLE II

Compound	Bond	Bond Energy in K. calories
CH_3—H	C—H	101·4*
C_2H_5—H	C—H	98·0
CH_2—H	C—H	85-3
CH—H	C—H	89·9
C—H	C—H	80
CF_3—H	C—H	103±4
CCl_3—H	C—H	88·9±3
C_6H_5—H	C—H	104
$C_6H_5CH_2$—H	C—H	77·5
CCl_4	C—Cl	67·9
CF_3Cl	C—Cl	83±3
CH_3Cl	C—Cl	80
$C_6H_5CH_2Cl$	C—Cl	88

* Walsh gives 104.

will be noticed: a change in bond energy with change in bond hybridization and a change in bond energy through interatomic forces in the same molecule. The importance of this has been shown by Walsh[29] who has correlated hybrid composition and other bond properties (Table II). From an analysis of these, Walsh concludes that

TABLE III

Molecule	Hybridization of bond	Force constant of C—H bond dynes/cm. $\times 10^5$	C—H Å	E (C—H) K. calories
CH	p	4·1	1·12	80
CH_4	sp^3	5·0	1·094	104
C_2H_4	sp^2	5·1	1·087	106
C_2H_2	sp	5·9	1·059	121

there is a reversal of the polarity of the C—H bond in this series. This conclusion was indicated earlier by Coulson[30] who obtains for the bond moments (C—H) 0.3D to (C—H) 0.7D. Any mechanism for a reaction involving the C—H bond will then have to take into account this reversal of polarity also. One has similarly to take note of interatomic forces as well. This is readily seen with the C—Cl bond energy values and their relationship to substitution reactions involving the C—Cl bond. These reactions also serve as illustrations of conditions in which the stabilizing influence of substituent groups on a positive charge on carbon can lead to a mechanistic change. The solvolytic reactions of alkyl halides in solution has been the subject of a very thorough study of the Ingold School,[31] and, as they are not directly of interest in the reactions of our present study, there is no justification in dealing extensively with them.

With the foregoing background, we are now in a position to proceed further into detailed analysis. Any reaction kinetics study should ultimately lead to a correlation between rate constant and molecular properties. In the present state of our knowledge, there is no prospect of a reasonably accurate theoretical calculation of rate constant by quantum mechanical methods. Even for the simplest molecules, calculationss of bond energies cannot be more accurate than 0.1 electron volt or about 2.3 k. calories per mole. Taking the Arrhenius rate equation this will make a difference in rate constant of more than a factor of 10 while experimental values are capable of considerably greater accuracy. However, using a system of harmonic oscillators and the vibration frequencies of the molecules, Slater[32] has been remarkably successful in getting closer values of the rate constants in a few unimolecular reactions, thereby showing an approach to the calculation of rate constants.

TABLE IV

Reaction constants from rate measurements

Reaction	Solvent	Temp. C	ϱ
$ArCOOH + MeOH + H^+ \rightarrow Ar\text{–}COOMe$	MeOH	25	—0·229
		40	—0·216
		50	—0·209
		60	—0·213
$ArCOOMe + OH^- \rightarrow ArCOO^-$	60% Acetone	0	2·460
		15	2·299
		25	2·229
		40	2·128
		50	1·920
$ArCOOC_2H_5 + OH^- \rightarrow ArCOO^-$	75% Ethanol	25	2·193
	85% Ethanol	25	2·537
	60% Acetone	25	2·265
	70% Dioxan	25	1·828
$ArCONH_2 + OH^- \rightarrow ArCOO^-$	H_2O	100	1·068
$ArCONH_2 + H^+ \rightarrow ArCOOH$	H_2O	100	0·119
$ArCH_2Cl + OH^- \rightarrow ArCH_2OH$	H_2O (OH⁻0·0506N)	30	—0·333
	(OH⁻0·104N)	30	—0·239
	(OH⁻0·259N)	30	—0·139

In spite of the limitations of an empirical generalization, however, where systematic structural changes in a reacting system are feasible as is the case with a number of aromatic molecules, a high degree of useful information is possible. We have noticed how the transition state method involves an equilibrium process involving the reactions. If the same molecule is involved in two similar equilibrium processes, one can reasonably conclude that the relative free energy changes will be affected in a similar way by substituents. Taking the classical paper of Bronsted and Pedersen[33] on acid-base catalysis, Hammett[34] has been able to demonstrate the relation between ionization constants of substituted benzoic acids and the influence of substituents on a variety of reactions. He puts forward the relation

$$\log \frac{k}{k_o} = \varrho\sigma$$

where k_o refers to the unsubstituted compound, ϱ is characteristic of the reaction and termed reactions constant and σ is characteristic of the substituent and known as the substituent constant. A collection of these constants for a few substituents in aromatic system is given in Table IV and Table V.[35]

TABLE V

Substituent Constants

Substituent Group	Substituent Constant ortho	meta	para
CH_3	—0·069		—0·170
C_2H_5	—0·043		—0·151
OH	—0·002		—0·357
OCH_3	+0·115		—0·268
OC_2H_5	0·150		—0·250
NH_2	—0·161		—0·660
$NHCH_3$	—0·302		—0·592
$NHCOC_6H_5$	0·217		0·078
COOH	0·355		0·265
$COOCH_3$	0·398		00·678
CN	0·678		1·000
NO_2	0·710		1·270
F	0·337		0·062
Cl	0·373		0·227
Br	0·391		0·232
I	0·352		0·276
C_6H_5	0·218		0·009

In every reaction where the influence of substituents are studied, three factors have to be considered; the transmission of electrical effects to the reaction region, the role of electron density at the reaction zone on the course of the reaction and the influence of temperature, solvent and other environmental conditions. The variations in the Hammett constants bring out this aspect very well. It should also be mentioned that ortho substituents do not fit in with the Hammett function. While attempts have been made to give a greater theoretical significance, the Hammett constants can at best be linked up only qualitatively with other molecular properties. The considerable deviations noticed in some instances strictly limit the applications even though the equation has been extended by Jaffe to poly substitution and heterocyclic compounds as well.[36] The tables give values to three places but it is doubtful if they can be depended on to this extent on account of the uncertainties involved.

Jaffe's tables bring out clearly another aspect of the Hammett functions, namely the influence of the concentration of the reactants, especially in solvolysis.

Any analysis of reactions in solution has to take into account initially the type of reactants. Broadly the following four divisions can be envisaged:

(i) Ionic reactions— { reactants of like charge / reactants of opposite charge

(ii) Ion dipole reactions— { with charge transfer / without charge transfer

(iii) Dipole-dipole reactions

(iv) Free radical reactions

Our present studies will be confined to a few reactions of the second and third categories.

Ionic reactions are generally quite complex and mechanistic studies require a wide range of experimental techniques to identify labile intermediates. Rigid control of ionic strength is a necessity and the range of solvents and, often, of experimental conditions are rigidly limited. The importance of the topology of the transition state is well brought out by a study of ionic reactions.

Reactions of the remaining categories, however, offer a wide range of experimental conditions, whether or environment or structure. These reveal a broad grouping together into two categories: "fast" reactions and "slow" reactions. The former category includes reactions between ions or between free radicals where the entropy of activation is small and the pre-exponential factor of the rate equation tends to have values of the right order of magnitude, viz., 10^{11} to 10^{13} for a bimolecular process. The majority of reactions, however, belong to the latter category and those which we have been studying invariably involve an appreciable entropy of activation. This is only to be expected while dealing with large molecules with a single reactive centre.

Where one or both reactants are ions, Brönsted has shown[37] that ionic strength of the solution is a factor to be reckoned with. A similar result was obtained by Bjerrum.[38] Working in regions where activities of ions can be expected to obey the Debye-Huckel limiting law, the expression

$$\log \frac{k}{k_o} = \frac{Z_A Z_B e^2 A \mu^{\frac{1}{2}}}{DkT} = 1{\cdot}02 Z_A Z_B \mu^{\frac{1}{2}}$$

for aqueous solutions at 25°C has been derived (k_o = rate constant at infinite dilution. A nearly quantitative verification has been observed in a number of instances but higher valence ions present difficulties which have necessitated the concept of specific ion interactions. This is also a trend indicated by some of the reactions in mixed solvents that will be considered in a later section.

The electrostatic model successfully used with ionic reactions has been extended to dipolar molecules by Kirkwood[39] using continum model for the solvent and a point dipole at the centre of a spherical molecule. This leads to a concept of the activity coefficient of the solute as a function of the dipole moment and of dielectric constant of the form $\frac{-\mu^2}{\gamma^3 kT} \frac{D-1}{2D+1}$. The equation is defective in not taking into account van der Waals forces which are of comparable magnitude. Laidler and Eyring are using this form for interpreting the influence of solvent on the reaction between polar molecules.[40] While quantitative correlations between rate constants and dielectric constant are possible, neither leads to any better understanding of the transition state.

The attempt of Moelwyn-Hughes to calculate the Coulombic energy of the transition state for an ion dipolar reaction[41] neglects polarization effects while the approach of Amis and Jaffe[42] using the Onsager model of a dipole surrounded by the solvent in evaluating solvent influences leads to complex expressions without any corresponding advantages.

Further, in dealing with systems where the dielectric constant of the solvent is low, there will be several additional complications. Formation of ion pairs, dielectric constant in the neighbourhood of the reaction zone differing from the bulk value, the existence of structure in the solvent especially where solvent mixtures are used, all give rise to deviations from ideal behaviour. A difficulty often met with in solution kinetics is the existence of concurrent mechanisms which are not influenced alike by the environment. In a few instances, it has been possible to choose experimental conditions so that one or other mechanism is so dominant and that no serious error is introduced by neglecting the other. This has enabled an understanding of the molecular processes but the method has its limitations and cannot be always used.

With this background we can proceed to a detailed study of three reactions where we have extensive data on both structural and solvent influences. The olefine-bromine reaction is an example of one between molecules where even the symmetrical molecule has to be polarized prior to reaction. Low dielectric constants lead to heterogeneous conditions and the mechanism was by no means clear when our work started.

The acid and alkaline hydrolysis of esters is one of the most extensively studied reactions and presents interesting aspects of the variation of the Hammett constants with environment, influence of "neutral salts", general acid-base catalysis and general topology of the transition state.

Oxidations of alcohols and aldehydes reveal situation where structural and solvent influences run counter to what current mechanisms lead us to expect and a different approach offers a better understanding of the problem.

A careful analysis of these in relation to other work enables a better understanding of the role of solvent but we are still far from evolving a quantitative picture.

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Lecture II

THE KINETICS OF THE OLEFINE BROMINE REACTION

Among the commonest reactions used in the laboratory for detecting the presence of an ethylenic bond in an organic compound is the addition of bromine. In spite of its being one of the oldest reactions, kinetic studies have been comparatively late. Like all reactions involving halogens, one has to look for both thermal and photochemical reactions. Compared to photochemical reactions involving chlorine, there are very few studies with bromine but what work has been done brings out both the similarities and differences.

Among the earlier work on photochemical addition of bromine should be mentioned those of Sudborough and Thomas[1] and of Plotnikow.[2] The early work of Hofmann and Kirmreuther[3] has to be considered essentially qualitative on account of uncontrolled catalytic conditions. Plotnikow's work on a variety of substituted ethylenes showed that the rate of bromination had a high temperature coefficient and depended on the concentration of reactants, solvents and intensity of light, varying as the square of the intensity. Plotnikow not only noticed the influence of substituents but also the formation of atomic bromine as a stage in the reaction.

Berthoud and his associates in a thorough study with cinnamic acid, stilbene and tetrachorethylene[4,5,6] notice that the rate of decrease of bromine concentration was proportional to I_o (Br_2) for strong absorption and to I_o $(Br_2)^{3/2}$ for weak absorption. Postulating a chain mechanism they suggest the following scheme:

Chain

$$Br_2 + h\nu \rightarrow 2\ Br \qquad I_{abs}$$
$$A + Br \rightarrow ABr \qquad \text{Fast}$$
$$ABr + Br_2 \rightarrow ABr_2 + Br \qquad \text{Rate determining step}$$

Chain Termination

$$ABr + ABr \rightarrow ABr_2 + A \text{ or } 2A + Br_2$$
$$ABr + Br \rightarrow ABr_2$$
$$Br + Br \rightarrow Br_2$$

These authors find the rate of addition to be the same with both trans cinnamic acid and stilbene and their results indicate an activation energy of 11 5 Kilocalories.

Bauer and Daniels[7,8] from a study of the reaction with cinnamic acid over the range 0° to 30°C postulated an energy chain mechanism, the chain initiation being the formation of active molecules by light, the subsequent steps involving an active addition product. Ghosh and his associates[9] consider a mechanism in which the formation of Bromine atoms but the active molecule is Br_3. This has to be ruled out on energy considerations. The Bauer-Daniels mechanism was revised later by Willard and Daniels[8] in their studies with tetrachlorethylene in which they take into account the inhibitory effect of the product of the reaction.

The greater part of the work on Bromine addition kinetics, however, has been on thermal processes. A proper analysis of these requires a consideration of the double bond. We have essentially to deal with two distinct pictures, one due to Pauling and the other to Huckel. In the first edition of Pauling's Nature of the Chemical Bond[10] he states "No general discussion of the orbitals involved in multiple bond formation analogous to that described for single bonds has been given. . . *It seems probable that the* orbitals involved in double bond formation by a carbon atom in a molecule such as ethylene are of the following type. The molecule is coplanar and of the four orbitals, s, p_x, p_y, p_z three lie in the plane of the molecule (taken as xy plane). From these three, s, p_x, p_y there can be constructed by linear combination three strong bond orbitals with their bond directions in the plane with which single bonds can be formed to the two hydrogen atoms and the other carbon atom. The p_z orbital, with lobes extending above and below the plane of the molecule, is then involved in the formation of the second half of the double bond." In the third edition of the same book,[11] *Pauling reverts to his earlier description*[12] with the two orbitals assumed to be essentially tetrahedral extending towards the corners of a tetrahedron defining a shared edge. This leads to a double bond description as involving two bent single bonds (Fig. 1). This is similar to the description of the classical organic chemists.

On the other hand, Huckel[13] divides the electron pair bonds into two types σ and π, *the former corresponding to the single* and bond of the usual type while the latter are formed from the p orbitals by lateral interaction The charge distribution of a π bond is on either side of the molecular plane and the π electrons are more easily excited on account of their space location The picture of the double bond on this description is given in Fgure 2.

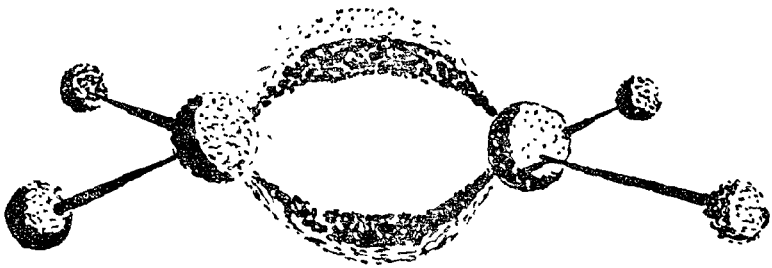
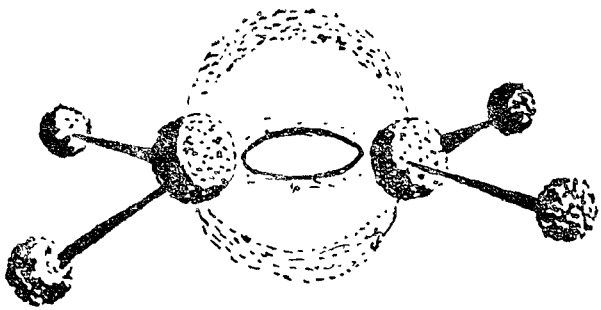


FIG. 1. Pauling's Bent Bond



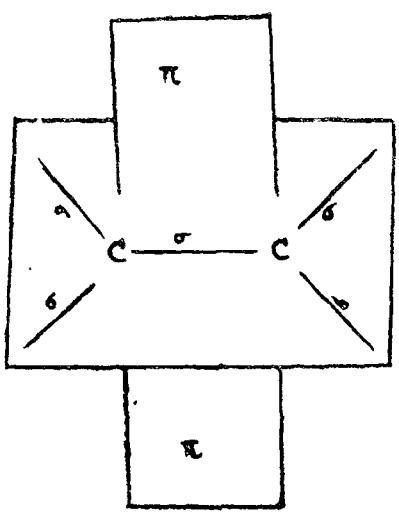


FIG. 2. Molecular Orbital Representation

Most theoretical approaches using the molecular orbital method bring out the Huckel picture as nearer the correct structure of ethylene. The trigonal sp^2 hybridization is necessary for a description of the ethylenic state. The C==C double bond consists of a pair of trigonal orbitals with a pair of π orbitals superposed. A consequence of this description is the need for maximum overlap of the p_z orbitals and a planar arrangement. The description also explains the restriction of free rotation while indicating a greater reactivity. Further, it should be mentioned that even using the valence bond method Penney[14] as well as Coulson[15] have been able to show that the energy of the π bond model is nearer the experimental value by 6 e.v. compared to the conventional strained model. In analyzing influence of substituents on the reactivity of the double bond, we have to bear in mind both the hybridization and bond polarity. Coulson has shown[16] how even in the C—H bond hybridization changes alter the polarity to the extent of even a reversal of charge. The state of polarization of the carbon atom must in its turn modify the approach of a polarized molecule.

Since the reactivity of the double bond is linked up with the polarizability to a considerable extent it is worthwhile examining the shift in the Raman line corresponding to a double bond and the classification of the groups in terms of the electrical shifts that can be envisaged on the basis of the electronic theory of organic reactions. The classification of the groups is presented in Table I while

TABLE I

Class		
1	+I	$:C<---CH_3$
2	−I	$:C--->\overset{+}{N}R_4$
3	−I−T	$:C--->COOH$
4	−I+T	$:C--->Br$
5	+I+T	$:C<--O^{\ominus}$
6	+I−T	$:C<---COO^{\ominus}$
7	±T	$:C---\phi$

the Raman frequencies are given in Table II. It is clear that there is a certain amount of correspondence between the nature of the substituent and the direction of shift of the Raman line. Since one of the contributing factors in the activation energy is the bond energy which in its turn is related to the characteristic frequency, a qualitative picture is possible as to the trend of reactivity if the nature of the interacting species is known.

TABLE II

Compound	Raman line corresponding to double bond cm^{-}
$H_2C{=}{=}CH_2$	1623
$H_2C{=}{=}CHCH_3$	1647
$RHC{=}{=}CHR$ cis	1669
$R_2C{=}{=}CH_2$	1654
$RHC{=}{=}CHR$ trans	1681
$R_2C{=}{=}CHR$	1679
$R_2C{=}{=}CR_2$	1689
$H_2C{=}{=}CHCl$	1608
$H_2C{=}{=}CHCOOH$	1637
$H_2C{=}{=}CHC_6H_5$	1631
HOOCHC==CHCOOH cis	
HOOCHC==CHCOOH trans	

From Y. K Syrkin and M. E. Dyatkina: Structure of Molecules and the Chemical Bond p. 174-176.

With this picture we are in a position to examine the kinetics of the olefine-bromine reactions in the thermal process in the absence of light. There has been very little study of gas reactions and though the first four members are available for study in the gas phase only ethylene has been thoroughly studied. The work of Gwyn Williams[17] has shown clearly the important features. The ethylene-bromine reaction in the gas phase is heterogeneous requiring a polar surface to initiate the reaction and the reaction is termolecular involving two molecules of bromine and one of ethylene.

Our interest here is primarily on the reaction in solution. Earlier work[18] has shown that the reaction involves a polarized bromine molecule in the initial step and can thus be expected to be favoured by a polar environment. As the rates of addition are widely dispersed, it was at first envisaged that a competitive method offers the best means of comparison. The success of the

method depends on certain basic assumptions. The reaction rate may be expressed in the form

$$\frac{dx}{dt} = k[\text{olefine}]\ [\text{Bromine}]^m\ [\text{Catalyst}]^n$$

The reaction is of the first order with reference to the olefine and *of the same order with reference to bromine and the catalyst or catalysts.* It is a tacit assumption that all the olefines follow the same mechanism. It is a pre-requisite of this approach that the reactivity of one olefine does not influence the reactivity of the other. If these conditions are satisfied then for any two substances X and Y the ratio of the rate constants can be seen from the integrated form of the equation to be given by the composition of the addition products. Using this method Ingold and Ingold[18] and Anantakrishnan and Ingold[19] obtained the results of Table III, former working at −35°C and the latter at −78°C, using methylene chloride as solvent. The work of Williams and James[20] showed that hydrogen bromide was a catalyst for the reaction in carbon tetrachloride and a careful examination[21] showed that this was the case for at least some of the compounds. The results reported in Table III were obtained under constant catalyst concentration.

TABLE III

$R_1R_2C{=}CR_3R_4$				Relative rate
R_1	R_2	R_3	R_4	
H	H	H	H	1
CH_3	H	H	H	2·03
CH_3	CH_3	H	H	5·53
CH_3	H	CH_3	H	3·7*
CH_3	CH_3	CH_3	H	10·4
CH_3	CH_3	CH_3	CH_3	14·0
C_6H_5	H	H	H	3·35
H	H	H	CHO	1·5
CH_3	H	H	CHO	3·0
CH_3	H	H	COOH	0·26

* Estimated value

One feature that has been noticed by most workers in the field is the presence of autocatalysis in many solvents[20,21,22,23,24]. Any mechanism that can be postulated for the reaction has to take into

account this aspect of the problem. A proper analysis of reaction mechanisms also require a systematic study of a number of compounds under varying conditions of temperature, concentration, structure and solvents.

The reaction in carbon tetrachloride which exhibited autocatalysis is further complicated by surface effects. Mathai[25] has shown that the rate of addition to olefine carboxylic acids is influenced by changing the surface from glass to silica or alumina. An examination of the system showed adsorption of both olefine and bromine. Crotonic acid showed a progressive retardation of the reaction while this was not the case with dimethyl acrylic acid. It was further noticed that while the addition of hydrogen bromide facilitated the reactions of crotonic acid, the addition of the dibromide served as a retarder. These observations clearly indicated the need for work under homogeneous conditions.

The functions of the product of reaction could be demonstrated in two ways: adding a known addition product or hydrogen bromide at the start of the reaction. Or starting a reaction and while it is in progress before the induction period is over, taking a measured quantity for addition to a fresh mixture. Both procedures revealed the variations with structure, solvent and temperature. A further clue in this direction was provided by the uncatalyzed addition to citraconic and maleic anhydrides[22] where reasonable rate constants could be obtained by using for the autocatalytic reaction using a rate equation of the form

$$\frac{dx}{dt} = kx(a+bx)^2 \qquad \text{with } b = -1$$

which leads to an integrated form

$$k = \frac{1}{t}\left(\frac{1}{a(a-x)} + \frac{1}{a^2}\log\frac{x}{a-x}\right)$$

The important role of a product of the reaction is thus to be considered an essential part of bromine addition kinetics.

Reference should now be made to two apparently divergent set of results. The school of Robertson and de La Mare have made extensive studies of the addition reaction at 24°C.[23,24] They find in general a third order kinetics and in a few cases postulate the formation of Br_4 molecules. It is a moot point whether the polarity of bromine will permit the formation of this tetrameric form. Our work has been mostly at temperatures above 30°C and the ole-

finic carboxylic acids gave good second order rate constants in the presence of added hydrogen bromide. In the case of dimethyl acrylic acid, the concentration of the catalysts needed was small and at higher temperatures indicated an approach to a constant value for second order rate constants. These results indicated a possible change of mechanism at some lower temperature and when the experiments were carried out 28°C, this became quite clear. We have thus to consider both bimolecular and termolecular mechanisms in understanding the addition kinetics of which the former is liable to catalysis while catalytic influence on the latter mechanism may be negligible. A further complication to be envisaged is the addition being initiated by a nucleophilic agent.

Since the reaction clearly showed the need for a polar environment, and in pure carbontetrachloride was heterogeneous, it was anticipated that a mixture of a polar solvent and carbon tetrachloride would enable a study of very fast addition reactions. Combining this with an obvious slowing down of a reaction of a higher order by dilution, reasonable data could be got for some of the olefinic hydrocarbons.

TABLE IV

Compound	Uncatalyzed Addition	Addition in the presence of HBr
$CH_2:CHBr$	0·0011	0·012
$CH_2:CHCH_2Cl$	1·6	3·8
$CH_2:CHCH_2Br$	1·0	2·2
$CH_2:CHCOOH$		0·44
$CH_2:CHCH_2OAc$	10	
$CH_3CH:CHCHO$		>10000
$Me_2C:CHCl$	0·5	0·3
$Me_2C:CHCOOH$	0·15	0·18
$HO_2CCH:CHCOOH$		0·56
$PhCH:CHBr$	0·11	0·07
$PhCH:CHCHO$		>10000
$PhCH:CHPh$ (trans)	18	8
$>C:C<$ (Ph, H; H, CH_2Cl)	77	16
$PhCH:CHNO_2$		ca 1·0
$PhCH:CHCOOH$ (cis)	0·063	0·07
p-$NO_2C_6H_4CH:CH$-$COOH$ (trans)		0·003
m-$NO_2C_6H_4CH:CHCOOH$ (trans)		0·006
p-$MeC_6H_4CH:CHCOPh$	7·8	
$PhCH:CHC_6H_3(NO_2)_2$ (2:4)	0·028	0·03

TABLE V

Olefine	Concentration of β-reactants	Temp °C	Catalyst and relative concentration	Rate constant	Reference
Trans crotonic acid	M/30	35	HBr; 11·1 mole%	0·0379	(32)
	M/80	24	HBr; 100 moles%	0·051	(23)
	M/40	30	$\overset{+}{N}Me_4Br$; „	$2·2\times10^{-4}$	(25)
Tiglic acid	M/30	35	HBr; 11·1 moles%	0·1995	(32)
	M/40	30	$\overset{+}{N}Me_4Br$ „	$4·8\times10^{-4}$	(25)
ββ-dimethyl acrylic acid	M/30	35	HBr; 11·1 miles%	0·778	(32)
	M/40	24	HBr; 100 moles%	0·18	(23)
	M/40	30	$\overset{+}{N}Me_4Br$ „	$6·5\times10^{-4}$	(25)

In discussing the effects of substituents so as to lead to a mechanism that can reconcile all factors, it will be worthwhile bringing together the different values of rate constants. These are presented in Tables IV, V and VI. Table IV gives the data of

TABLE VI[28]

Solvent	Concentration	Compound	Relative rate
Acetic acid	M/40	trans crotonic acid	1·00
		Tiglic acid	5·00
		ββ-Dimethyl acrylic acid	45
		Maleic acid	1·10
		Fumaric acid	0·05
		Maleic Anhydride	(0·15)*
		Citraconic Anhydride	(0·18)*
60% CH_3COOH	M/80	Allyl Chloride	1·40
40% CCl_4		Allyl Bromide	(1·00)*
		Methallyl Chloride	43·00
		Allyl Propionate	29·00
40% CH_3COOH	M/400	Styrene	154·00
		trans Stilbene	1·00
		Triphenyl ethylene	17·00
		Tetraphenyl ethylene	0·00
		Cyclohexene	(4500 circa)*

*Estimated values.

Robertson and his associates.[23,24] Direct comparison of the results of the two schools of workers is not possible on account of the different experimental conditions. However, certain broad results are to be found in common. Electrophilic initiation of attack is facilitated by the presence of substituents in class I (vide Table I) unsymmetrical substitution being more effective than symmetrical. Substituents of class III are more deactivating than those of class IV. A substituent of class 7 can be activating but resonance effects make a symmetrical substitution neutralize the activation. This is not the case with class 1 substituents. Where several substituents are involved, the overall dipole moment and the resulting polariza-

bility of the double bond have to be taken into account. The existing data are inadequate for a quantitative picture of this aspect. It appears clear that the introduction of an "electron sink" like the carbonyl or nitro group functions as a powerful deactivator for electrophilic initiation of the addition reaction and if close enough to the double bond, can induce nucleophilic addition. Transmission through a phenyl group is inadequate to bring about this change. These observations are consistent with the earlier expectations from competitive studies.

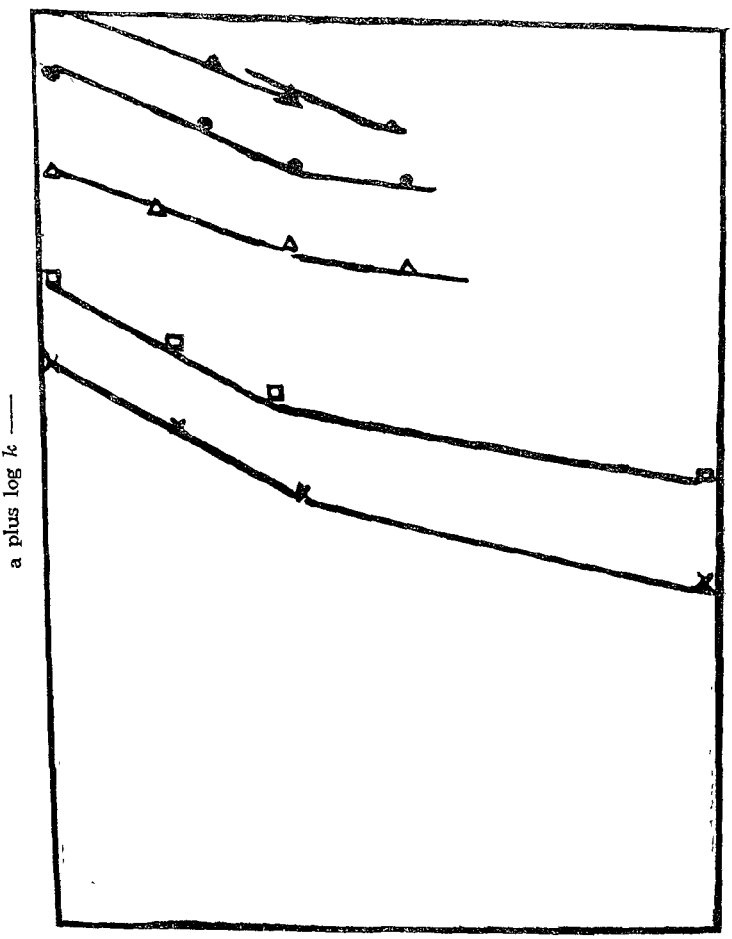


FIG. 3 log k—1/T curves see Ref. 32

TABLE VII

Compound	Solvent	Concentration of reactants	Activation Energy between 28°—35°C	Activation Energy between 35°—40°C	$K_3 \times 10^3$
Allyl Chloride	100% HAc	M/80	2·82	0·87	0·87
	40% HAc	M/80	1·07		0·013
	60% HAc	M/80	1·08		0·003
Allyl Bromide	100% HAc	M/80	3·04	3·29	1·30
	80% HAc	M/80	3·00	3·94	0·51
Allyl Propionate	80% HAc	M/160	5·21	9·51	$7·6 \times 10^3$
	100% HAc	M/320		12·43	$9·3 \times 10^4$
Crotonic acid	100% HAc	M/15	<3·0*	10·8	$1·952 \times 10^3$
	100% HAc	M/30	<3·0*	10·8	$1·952 \times 10^3$
Dimethyl Acrylic acid	100% HAc	M/30		4·50	44·3
	100% HAc	M/60		7·2	
Fumaric acid	100% HAc	M/30		21·3	$2·66 \times 10^9$

* Below 25°C.

Using only the rate constant as a comparable quantity, is completely misleading. As is well known, the rate constant is a composite quantity involving the activation energy and an entropy term included in the pre-exponential part of the Arrhenius rate equation. Whatever form is assumed for the transition state, the bond energy will be involved and this will depend on the evironment. The entropy term will incorporate a steric factor. The rate constant can be used for comparison only when the pre-exponential term can be taken to be identical. This cannot be the case. While molecular collisions have no directional preference, only encounters in the region of the double bond can lead to reaction. This will make itself felt in the entropy term. A better guide to structural influences is an analysis of the Arrhenius parameters. An essential feature of termolecular reactions is a low activation energy and a low temperature coefficient. A bimolecular reaction involves a higher value for both pre-exponential term and activation energy leading to a larger value for the temperature coefficient. It is well known that one of the diagnostic criteria for a change of mechanism is provided by changes in the slope of the lok k—1/T graph[29] and this criterion can be applied to the present system also. The variations in the Arrhenius parameters are indicated in Table VII.[30] The change in slope of the log k—1/T curve for a few compounds is shown in Figure 3.

Since activation energy can be expected to be susceptible also to environmental influences and since qualitatively it is known that a polar environment favours the addition process, it is worthwhile examining the influence of the dielectric constant. It will be shown later that using the bulk dielectric constant is not always a reliable criterion, but in the absence of any precise estimate of this quantity in the neighbourhood of the reaction zone, this has

TABLE VIII

Compound		Dielectric Constant of Solvent			
		6·24	4·85	3·725	2·968
Allyl Propionate	E	12·43	7·36	4·69	2·74
	$\log_{10}$ PZ	7·97	6·88	4·54	2·64
Stilbene	E		5·43	5·52	5·34
	$\log_{10}$ PZ		5·63	5·16	4·49

to serve the purpose. This can be seen from the results presented in Table VIII. The rise in value of the two parameters with increased dielectric constant is significant especially in the first example. The influence on the composite rate constant can also be equally revealing (Table IX).

TABLE IX

Dielectric constant of solvent	Rate Constant	
	Allyl Chloride M/80 solution	Stilbene M/200 solution
6·24	456·3	11,060·0
5·45	273·4	
4·85	137·4	3,698·0
3·725	34·8	1,074·0

A feature of the reaction studied is an abnormally low value for the pre-exponential factor of the rate equation. As Eyring and his associates have pointed out[32] several factors can be listed that modify this quantity:

(1) The necessity of correct orientation of reactant molecules can require a factor of 10^{-2} to 10^{-4}.[31]

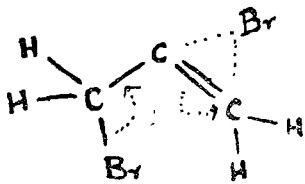
(2) Since the energy has to be associated with the appropriate vibration phase, this will need a correction of 10^{-2}.

(3) The repulsive forces or steric hindrance will lead to a factor 10^{-1} or more.

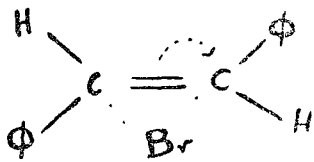
(4) Solvation of the reactants and the requisite spatial distribution will require a correction term of 10^{-2}.

Thus the pre-exponential term instead of a normal value of the order of 10^{13} for a bimolecular reaction or 10^{9}–10^{11} for a termolecular reaction can go down to 10^{2}–10^{4}, depending on the extent to which each of these factors operate. In addition the symmetry properties of the transition state will also play its part. This will be particularly seen in compounds like allyl bromide and trans diphenyl ethylene. In the case of tetraphenyl ethylene, repulsive forces can be quite large and leads to a negligible value for PZ and the double bond is so hedged in that addition is precluded (Figure 4).

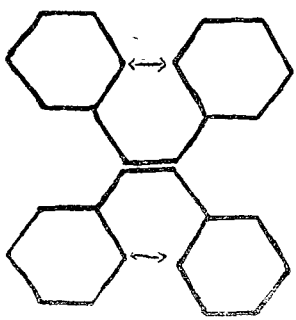
Allyl Bromide



Sym. Diphenyl Ethylene



Tetraphenyl Ethylene



Nonplanar molecule

Double bond of lower bond order

FIG. 4. Symmetry and steric properties in relation to the Transition State

One other aspect of the correlation between the Arrhenius parameters should be mentioned. It has been observed by Fairclough and Hinshelwood[33] that there is a general uniformity in the trends of the two and often log k—$1/E^{1/2}$ graph is linear, though deviations are not unknown. The results with bromine addition provide us with examples of the three types expected:

(1) Both PZ and E change in the same direction
(2) E is constant but PZ changes
(3) PZ is constant but E changes

The first type occurs when the time for attainment of right phase for reaction is very much larger than the interval between activation and deactivation. The second type of change is noticeable when all the factors mentioned in an earlier paragraph are operative and an increase occurs when the transition state is more sol-

vated and also when the interval between activation and reaction gets reduced.

In analyzing the mechanism of the reaction, one of the factors required to be known is whether we are dealing with a reaction between dipoles or whether we have any ionic intervention in the rate determining step. The bromine molecule does not exhibit any polarity unlike iodine and if polymeric molecules are to participate, the reaction should show any correlation with the dielectric environment. Extending the Debye-Huckel-Onsager theory of interionic attraction, Kirkwood[34] has shown that for a dipole reaction there should be a linear relationship between log K and $D-1/2D+1$. An examination of the data shows that while generally this is noticed in all the compounds studied, in the region of lowest dielectric constants, deviations from linearity are quite pronounced. Apparently, in these regions deactivation takes place before the appropriate orientation in phase can take place. Also, since the reaction appeared to involve a greater degree of solvation in the transition state, with a non-polar solvent no serious difference in the extent of solvation is present.

Before we can proceed to a consideration of the reaction mechanism, it is necessary to consisder one other aspect. We have noticed that dipole moment as well as symmetry properties modify the reaction rate. It is commonly stated in textbooks that the cis form of an olefine reacts faster than the trans form.[35] It is also recognized that addition invariably is to the trans positions. Both these need careful testing. Fumaric and maleic acids can be used for the purpose. Here, Br^+ can initiate isomerization of the cis to the trans form while Robertson[36] considers the possibility of nucleophilic attack. While the cis form reacts faster, a definite amount of the addition product corresponding to the trans form is invariably obtained. Comparing angelic and tiglic acids, Buckles and his associates[37] find that the latter reacts faster and preliminary observations indicate that trans crotonic acid reacts faster than the cis form.

In postulating a mechanism we have then to consider the following factors:

(i) Transmission of electrical effects to the reaction zone
(ii) The susceptibility of the reaction to such changes in electron density
(iii) Effect of temperature
(iv) Effect of solvent
(v) Topology of the transition state.

Summarizing the results obtained in relation to these factors we can make the following observations:

(i) The reaction requires a polar environment for homogeneous conditions

(ii) The majority of the systems studied indicate initiation of an electrophilic attack by a polarized molecule of bromine

(iii) The reaction has the characteristics of one between dipoles

(iv) There is a mechanistic change with temperature and change in concentration

(v) Non-polar solvents, lower temperature, higher concentrations and deactivating groups attached to the ethylenic centre facilitate termolecular addition

(vi) Polar solvents, low concentrations, higher temperatures and activating groups facilitate bimolecular electrophilic addition

(vii) The substituent groups may or may not lead to change in configuration

(viii) Bimolecular addition is susceptible to catalysis but not termolecular

(ix) The uncatalyzed reaction generally shows autocatalysis and rate constants can be obtained from the appropriate rate equation. Instances of retardation by products are also present.

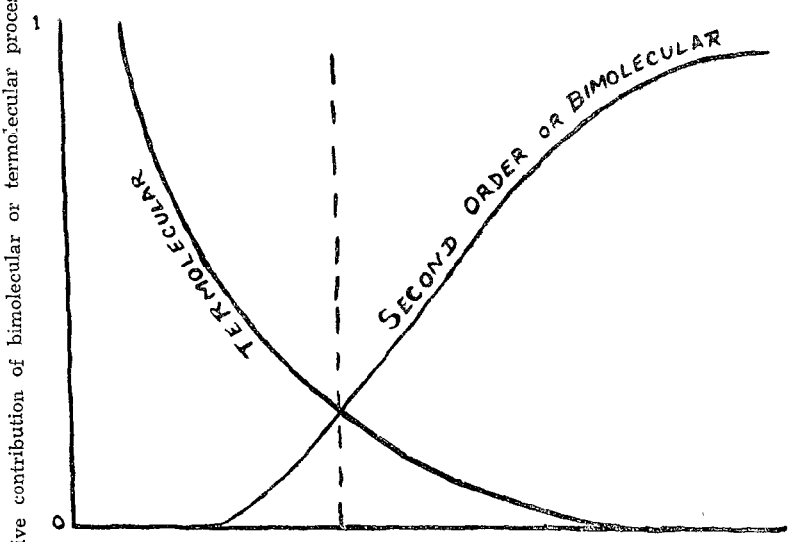


FIG. 5. Activation by Polarity of Solvent

TABLE X

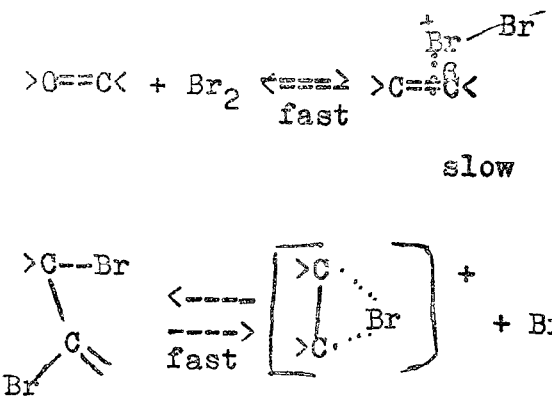
Class of Substituent group	Mechanism preferred	Catalysis	Configuration change
+I	Electrophilic, Bimolecular	Small effect	Possible
+I–T	Electrophilic, Bimolecular and termolecular	Pronounced	
–I+T	Electrophilic, Bimole-cular and termolecular	Pronounced	
–I–T	Nucleophilic, α:β acid can be electrophilic, Bi- and termole-cular	Small effect; termolecular uncatalyzed	None
+I+T	Electrophilic, bimolecular	Negligible	Possible
+T			
–T	Nucleophilic, possibly termole-cular	None	None
–I	Nucleophilic, Bimolecular	None	None

The net result of all these factors is that we are very often confronted with a concurrent bimolecular and termolecular process. The substituent groups can be classified into suitable groups as indicated in Table X[38] and a qualitative picture of the overall process show graphically (Figure 5).

Work done so far has not extended to all categories of substituents but the broad trend is noticeable.

Except where there is an accumulation of activating groups or a strong electron sink one has to postulate a mechanism envisaging concurrent processes.

The starting point for the mechanism is that of Roberts and Kimball.[39]



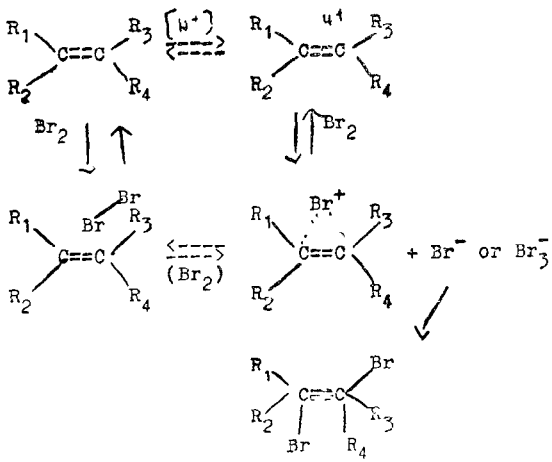
This can be the picture for a bimolecular uncatalyzed process. The essential feature is the formation of a molecular complex between the olefine and bromine which is then transformed into the 'onium' compound and then to the dibromide. The linking of the Br^+ cation with the π electrons of the olefine accounts for both the steric character of the addition and the electrophilic initiation of attack.

It has been noticed earlier that the bimolecular process may be catalyzed by hydrogen bromide. This is not inconsistent with the formation of the π complex. Association between H^+ and the π electrons of the olefine has been established by Winstein and Lucas[40] and the double bond activated thus is more liable to attack by the halogen, with bromonium ion taking the place of H^+ which is released for activation of other molecules. The configurational studies of Winstein and his associates[40] also provide the evidence for such a stage.

The termolecular mechanism is also easy to visualize. An encounter of the π complex with another bromine molecule leads to the 'onium resonance hybrid and a Br_3 which reacts subsequently with formation of Br_2 and the olefine dibromide. Where structural activation of the double bond is inadequate and hydrogen ion catalysis small, this step will acquire greater importance.

The need for formation of a π complex prior to the addition reaction readily accounts for the relative inactivity when bulky groups are attached to the olefine, steric repulsions becoming quite significant.

The concurrent bimolecular and termolecular processes can then be visualized thus:



There is a tacit assumption that the rate determining stage is the formation of the resonance hybrid. Structural factors can, however, lead to conditions when it is no longer justifiable to consider any single stage as the rate determining. Under such conditions, the system can show very complex kinetics as is found to be the case. At low temperatures, higher concentrations and solvents of low dielectric constants, the termolecular process is possible as the π complex initially formed can be stable long enough for the encounter with a second bromine molecule. On the other hand, higher temperatures, solvents of higher dielectric constant and low concentrations favour tle bimolecular process. As there is no single dominant factor, the net result is a complex system of concurrent reactions at intermediate regions. However, the cumulative influ-

ence of activating groups as with the methyl ethlene can make the bimolecular process as the only mechanism.

A clue to the mechanism is provided by the influence of the dielectric constant on the rate. If a polarized bromine molecule is involved in the initial step, we can expect following Kirkwood[34] a linear relation between log k and $\frac{2D+1}{D-1}$. Such a relationship is actually observed with all the compounds studied[41] using a mixture of glacial acetic and carbon tetrachloride under conditions where there is no deviation from homogenous behaviour. In the region of low dielectric constants, the more reactive compounds show departures from linearity. This aspect will be discussed in a later section.

We can then conclude that the olefine-bromine reaction is one which involves the following stages:

1. The reaction between the olefine and a polarized bromine molecule forming a π complex. This may be assisted in some compounds by hydrogen ions.
2. The π complex changes over to the bromonium resonance hybrid (Roberts and Kimball[42]). This may involve a second bromine molecule.
3. The resonance hybrid changes over to the product. The last stage is likely to be fast and the molecularity of the reaction is decided by the relative importance of 1 and 2 in the rate determining step. An uncertain feature is the extent of solvation of the transition state. As the only entity carrying a positive charge we can visualize this to be more solvated than either the reactants or products and abnormalities in solvent influences can arise through changes in the solvation. In the present state of our knowledge, it is difficult to predict in advance which stage is the rate determining step. In the absence of a clear-cut position any rate equation used in interpretation of data has to be considered subjective.

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Lecture III

KINETICS OF ESTER HYDROLYSIS

The hydrolysis of esters of carboxylic acids have been extensively studied because of the variety of problems presented. The reaction is one of the most thoroughly investigated for acids and base catalysis. Besides, the ready availability of a number of closely related structures makes for a systematic study of structural and solvent influences. The reversibility of the acid catalyzed system is an interesting complication.

Warder[1] had noticed that alkaline hydrolysis was kinetically of the second order while Reicher[2] observed the dependence on hydroxyl ion concentration. de Hemptine[3] noticed the greater catalytic effect of hydroxyl ion compared to the hydrogen and further made the significant observation that the acidic component of the ester was more effective in structural influences.

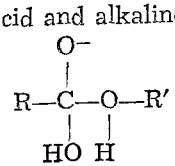
The early dualistic theory of catalysis was discarded in favour of a more accurate description that became possible after Bronsted[4] introduced the concept of general acids and bases. A hydrolytic reaction taking place under these conditions can thus be represented by a rate equation of the form

$$\frac{dx}{dt} = k_a[OH_3]^+\underline{|S|} + k_b[OH]\underline{|S|} + k_c[H_2O]\underline{|S|}$$

for a reaction in aqueous rolution. Dawson[5] investigated the relative importance of each, but his results are not free from complications and do not give unambiguous information.

Solvolysis of esters is a convenient reaction for study of both structural and solvent influences under homogeneous conditions. An early study by Cashimore, McCombie and Scarborough[6] in alcohol-water mixtures showed a trend in velocity with different bases following the stability of the alkoxides and the equilibrium concentartion of alkoxide ion.

The first important study on the mechanistic side has been that of Lowry.[7] The significance of this is the postulate of a common intermediate for both acid and alkaline hydrolysis of the form



The uncertainties in the mechanism of ester hydrolysis were ultimately clarified by Ingold and his associates[8] and extended by Frost and Pearson.[9] As a result of this, eight different modes are possible (Table I) for the rate-determining step. Mechanisms I

TABLE I

I. Acyl oxygen fission

(i) $R-C(=O)-\overset{+}{O}H-R' \rightleftharpoons R-C^{+} + R'OH$ A_1

(ii) $H_2O + R-C(=O)-\overset{+}{O}H-R' \rightleftharpoons R-C(=O)-OH + R'OH$ A_2

(iii) $R-C(=O)-OR' \rightleftharpoons R-\overset{+}{C}=O + R'O^{-}$ B_1

(iv) $R-C(=O)-OR' + OH^{-} \rightleftharpoons R-C(=O)-OH + OR^{-}$ B_2

II. Alkyl-oxygen fission

(i) $R-C(=O)-\overset{+}{O}H-R' \rightleftharpoons \overset{+}{R'} + R-C(=O)-OH$ A'_1

(ii) $R'-\overset{+}{C}(=O)-OH-R' + H_2O \rightleftharpoons R'-\overset{+}{O}H_2 + R-C(=O)-OH$ A'_2

(iii) $R-C(=O)-OR' \rightleftharpoons R-C(=O)-O^{-} + R'^{+}$ B'_1

(iv) $R-C(=O)-OR' + H_2O \rightleftharpoons R'-\overset{+}{O}H_2 + R-C(=O)-O^{-}$ B'_2

ii and I iv are commonly met with using simple esters under acidic or basic hydrolytic conditions. Evidence for I i process has been provided by Traffers and Hammett[10] while Kenyon and his associates[11] using esters of allyl alcohol and phthalic acid demonstrated mechanism II i. The possibility of a unimolecular alkyl-oxygen fission under weakly alkaline conditions is indicated by the hydrolysis of triphenylmethyl benzoate studied by Hammond and Rudesall.[12] It may be possible to decide *a priori* whether a reaction proceeds by acyl-oxygen fission or alkyl-oxygen from an analysis of the characteristic frequencies. Since an essential part of the activation energy of a reaction arises from the stretching of the bond undergoing rupture, the Raman frequency of the corresponding bonds may be expected to provide the clue. We find that a linear correlation is noticeable between the square of the carbonyl frequency (expressed in wave numbers) and the activation energy for the hydrolysis.[13] In making the comparisons, it is necessary to use data in a single solvent. The need for this can be seen from the data in Table II. The parallel trend is noticeable in any one

TABLE II

Hydrolysis of ethyl esters of substituted benzoic acid

Substituent	$+10^{-6}$	Acid Hydrolysis Ethanol 85%	60%	Alkaline Hydrolysis 85% Ethanol
p-Nitro	2·958	14·50	17 52	12·40
p-Iodo		16·70		
p-Bromo	2 924	16·80	18·95	
p-Chloro	2·934	16·80	19 01	
H	2 906	17·70	19 63	14 56
p-Methyl	2·889	18 20	19·76	15 16
p-Methoxy	2·874	19·65	20 79	
p-Amino	2·856	20·00		16 70

solvent. The influence of the solvent as such will be considered in a later section. Though the carbonyl bond as such is not involved, none the less, its influence has to be expected in the rate-determining stage of acyl-oxygen fission, but not in alkyl-oxygen fission. We can then conclude that in the compounds considered, the mechanisms necessarily involve an acyl-oxygen fission in both acid and alkaline hydrolysis.

In analyzing the influence of substituents an interesting empirical correlation has been reported between the group dipole moment and the influence on the apparent activation energy. Nathan and Watson[14] find a relation of the form

$$E_s = E_u + K_1\mu + K_2\mu^2$$

satisfactory. Moelwyn-Hughes[15] does not consider the quadratic term which takes into account the polarity induced at the site of attack by the dipole as significant and reports reasonable agreement between calculated and observed values within 0·5 kilocalories. It is difficult to agree with this author's conclusion, that there is no need for the quadratic term. It is well known that the dipole moment of any aromatic compound with two dipoles has to reckon with induced moments and any coincidence of the type mentioned by ignoring this can at best be fortuitous.

TABLE III

Acid Hydrolysis of some esters of aliphatic acids

Dissociation Constants (18)	Ester	Activation Energy of the reaction in	
		Dioxan 60% (16)	Acetone 60% (17)
4·76	Ethyl Acetate	16·93	
2·86	Ethyl Chloracetate	16·64	17·00
1·29	Ethyl Dichloracetate		14·75
2·86	Ethyl Bromacetate	15·78	
3·12	Ethyl Iodacetate	14·23	
4·88	Ethyl Propionate	15·96	
2·80	Ethyl α-Chloroproionate	15·06	
4·08	Ethyl β-Chloropropionate	16·07	
2·91	Ethyl α-Bromopropionate	16·83	
4·02	Ethyl β-Bromopropionate	18·69	
(ca 3?)	Ethyl α-Iodopropionate	17·26	
4·06	Ethyl β-Iodopropionate	16·23	
	Ethyl Formate		15·60
	Methyl Formate		15·60
4·31	Phenyl Acetate		16·7 (17·2)
	Methyl Acetate		16·25
	Benzyl Acetate		16·40

A study of aliphatic esters will be a convenient one for analysing the influence of structure before proceeding to a fuller analysis of aromatic compounds. Here too, variations are noticeable with solvents and any comparison has to be only with a single solvent system. The available data for the purpose are collected together in Table III and Table IV.

TABLE IV

Alkaline Hydrolysis of esters of aliphatic acids (19)

Ethyl Ester of Acid		E
Acetic	..	14·20
Propionate	..	14·50
n-Butyric	..	15·00
n-Valeric	..	14·70
n-Hexoic	..	14·80
n-Heptoic	..	15·00
n-Octoic	..	15·00
iso-Butyric	..	14 50
iso-Valeric	..	15·70
Methyl Ethyl Acetic	..	15·40
Diethyl Acetic	..	16·50
Trimethyl Acetic	..	17·40

In analyzing the influence of substituents on the hydrolytic reaction, dipole moments of the compounds and dissociation constants may be expected to give some indication. Paucity of data rules out the first. It will be observed from the tables that there is no correlation between dissociation constant and the activation energy for ester hydrolysis. This is only to be expected since this property may show a similar trend only if we are dealing with alkyl-oxygen fission. We have thus an indirect indication that in all the simple aliphatic esters, we are having only mechanisms i and ii of Table I to be considered for acid hydrolysis and iv for alkaline hydrolysis. The sharp reduction in the activation energy noticed with ethyl dichloracetate possibly indicates a mechanistic change from the mono derivative. The cumulative effect of this chlorine facilitates mechanism (i), a unimolecular process as the rate-determining step.

A substituent can influence the rate by modifying the activation energy or the pre-exponential factor in the Arrhenius rate equation or possibly both may be affected. In a series of homologues or substitution products, if the pre-exponential term is un-

affected, one can expect a linear correlation between E and log k with points on a line having the slope $-2 \cdot 303R$. We find on examination of the relevant data[20] that no such behaviour is observed. In the aliphatic series, then, we have both parameters affected by substituents.

In a careful analysis of the conditions for fission, Kenyon and Davies[21] find that with the alcoholic component as a primary or secondary alkyl derivative the carbonyl carbon is more liable to a nucleophilic attack. Further hydrolysis under neutral or basic conditions generally favours acyl-oxygen fission. They make the interesting observation that in a highly polar solvent, unimolecular attack is susceptible to acid catalysis if the attacking agent is only weakly nucleophilic, the acyl group is linked to an electron-attracting and the carbonyl is linked with an electron repelling substituent.

In the homologous series, the activation energy tends to a constant value from the 4-carbon acid, but the effect of branching is irregular and needs a re-examination. Similarly, the influence of halogen in the acyl component also does not show any regularity and a re-examination is called for.

For a more convenient group of esters where substituent effects could be analyzed, we have to turn to esters of aromatic acids or to benzyl esters of acetic and other acids. Again we have work in different solvents and comparison has to be only amongst the results in the same solvent. We can consider the observations of Hinshelwood and coworkers[22] and our own[23,24] for this purpose. These are presented in Tables V, VI, VII and VIII.

TABLE V

Influence of substituents on the alkoxy group

	Alkoxy group	Alkaline E	Hydrolysis $\log_{10}PZ$	Acid E	Hydrolysis $\log_{10}PZ$
Esters of Acetic acid	Benzyl	12·32	7·91	16·40	7·58
	m-methyl benzyl	11·65	7·91	16·10	7·29
	m-nitro benzyl	12·42	7·88	16·20	7·35
	Phenyl	12·55	8·97	17·20	8·10
	m-Tolyl	12·86	9·04	17 20	8·08
	m-nitro phenyl	11·31	9·06	16·70	7 61
	p-methyl benzyl	12·48	7·88	16·20	7·40
	p-Nitro benzyl	11·47	7·87	16·20	7·39
	p-Tolyl	12·89	8·99	17·2	8·09
	p-nitro phenyl	11·00	9·00		

TABLE VI
Esters of benzoic acid and cinnamic acid

Ester		E	△H	$\log_{10}$PZ	△S
Ethyl Benzoate		12·23	12·618	7·600	—23·84
Benzyl Benzoate	a	12·79	12·178	7·220	—25·59
	b	13·96	13·346	7·964	—22·17
Ethyl Cinnamate	a	13·22	12·608	7·59	—23·89
	b	14·44	13·828	8·36	—20·34
Benzyl Cinnamate	a	12·53	11·918	7·35	—25·11
	b	14·20	13·588	8·42	—20·08

TABLE VII
Influence of substituents in the acyl group
(Ethyl esters of benzoic acid)

Acid	60% Acetone-Water		60% Dioxan-Water	
	E	$\log_{10}$PZ	E	$\log_{10}$PZ
Benzoic	14·56	8·18	13·23	7·60
m-methyl benzoic	14·87	8·17		
m-amino benzoic	14 98	8·25		
m-Nitro benzoic	12·80	8·56		
o-nitro benzoic			11·61	7·07
p-Nitro benzoic	12·40	8·55	11·57	8·10
p-methyl benzoic	15·16	8·22		
p-amino benzoic	16·70	8·23		

TABLE VIII
Influence of substituents on Acid hydrolysis
in 60% acetone-water system

Ester		E	$\log_{10}$PZ
Ethyl benzoate	..	20·25	7·84
p-methyl	..	20·42	7·88
p-methoxy	..	20·95	8 07
p-hydroxy	..	20 73	7·83
p-chloro	..	20·19	7·72
p-bromo	..	20·12	7·72
p-Nitro	..	19·65	7·57
o-Nitro	..	21·10	7·22
m-Nitro	..	20·10	7·73

In analyzing the influence of substituents, one of the commonest approaches is the use of the Hammett constants ϱ and σ which are linked up with the rate constants. The review by Jaffe[25] brings out clearly the variable nature of these functions while Taft and his associates[26,27] have attempted to interpret the substituent constant in terms of the inductive and resonance effects. An elaborate system of calculation based on a linear inductive relationship of the form $I = \sigma$, ϱ, and linked with an aliphatic substituent constant has been used for evaluating the inductive part of the effect of substitution. Resonance effects have been evaluated using the relationship

$$\log \frac{k^m}{k_o} = I + R^m \text{ and } \log \frac{k^p}{k_o} = I + R^p$$

with the further assumption $(R^m/R^p) = \alpha$, a constant. The $^\sigma R$ scale thus built has been reported satisfactory for meta substitution but not for para substitutions.

All methods of comparing substituent effects based on the rate constant cannot be expected to lead to a satisfactory result. While recognizing the limitations of the Arrhenius rate equation or while using the rate equation on the basis of the transition state method, it is clear that the rate constant is a composite term. One has to separate out the experimental activation energy and the pre-exponential term or the heat of activation and entropy of activation term. Even though the two quantities may not be absolutely independent, it is not necessary that substituents modify these to the same extent. This will be readily seen from the data foregoing tables.

The pre-exponential term is dependent on several factors: thermal changes in forming the transition state, reaction mechanism, stringent orientation of reactants, deactivation by solvent and repulsive forces, all have a part. It is difficult at present to apportion the magnitude of each of these factors. A certain degree of comparison is however possible, especially with the values of activation energy.

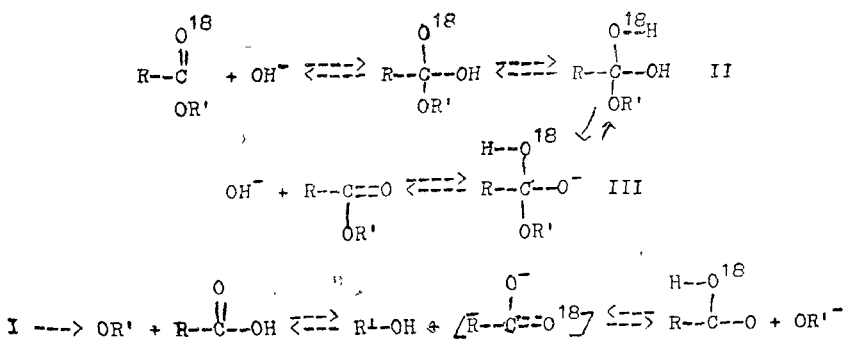
Evans and Polanyi[28] have shown that a relationship of the form

$$T\triangle S = \alpha\triangle H + \beta$$

holds good in a series of closely related structures and Laidler and Eyring[29] have shown this to be applicable,

The aliphatic and aromatic series present an interesting contrast in several respects. The aliphatic esters show no correlations between changes in activation energy and changes in the pre-exponential term. This lack of correspondence has also been noticed by Grace and Symons in their study of esters of branched chain alcohols.[30] It is of interest to note that while the branching chains on the acyl part leads to an increased activation energy for the bimolecular process, the branching on the alkyloxy part leads to a reduction of the activation energy. This is only to be expected for an acyl-oxygen fission. The aromatic esters, on the other hand, show the correlation between the two parameters of the type postulated by Fairclough and Hinshelwood.[31] It is generally found that changes in the structure of the alcoholic component has only a small effect on the activation energy but substitution in the acyl part has a more pronounced effect. In contrast to the aliphatic series, in the aromatic system, there is very little change in the pre-exponential term so that the primary effect of the substituent appears to be on the activation energy.

Mention should be made here to the work of Bender and his school on the mechanism of ester hydrolysis using isotopic oxygen and measuring the rates of hydrolysis.[32] The mechanism postulated is one which involves an intermediate of the aldehyde hydrate type as indicated below:



On this basis of loss in isotopic oxygen by the ester during hydrolysis, Bender considers that an S_Ni type of mechanism is not feasible. In the initial approach Bender considered that proton transfer rates need not be taken into account, but in his later work[33] he finds that it is necessary to take this also into account. In their analysis, there is the tacit assumption that the pK_a of the postu-

lated intermediate is of the same order of magnitude as that of formaldehyde hydrates. This is by no means justifiable. There is also no valid reason why the exchange and hydrolytic reactions should proceed by the single intermediate. It is equally possible that the two are concurrent mechanisms. The mechanism also fails to account for alkyl-oxygen fission which has been established by Kenyon and his associates[34] as well as by Hammond and Rudesall.[35] A further difficulty in the postulate of the dihydroxy intermediate by proton transfer is its unreality. Even in the case of acetonyl acetone, the proportion of enol is considerably reduced in water. In alkaline solutions, formation of an enolate by the carbonyl compounds is the slow rate-determining step in reaction. A different interpretation has then to be sought for the isotopic exchange reaction. Bender has further criticized the transition state postulated for the SN2 mechanism or Dewar's picture involving the π orbital of the carbonyl group in a resonance-stabilized transition state.[37] Bender's preference for an alkyl-oxygen involvement is not necessarily a better one. As will be noticed later, the solvation of the transition state indicates a more symmetrical charge distribution than is possible by Bender's mechanism. Similarly, one cannot accept without further evidence the postulate of an intermediate anhydride in the hydrolysis of methyl hydrogen phthalate.[36] Similarly, intramolecular hydrolysis in which migration of an acyl group is postulated needs further study with systematic structure variations.

While a good amount of work has been done with monocarboxylic esters, it is only comparatively recently that esters of polybasic acids have been studied. The separation of the first and second constant from a single analysis has been a stumbling block. The solution of the differential equation for consecutive second order reactions involves the ratio of the rate constants. Neglecting, as a first approximation, the second step, by a series of approximations, Ingold[38] deduced that

$$t = \frac{a-k}{ka^2}\left\{1 + \frac{a-k}{a}\left(1 - \frac{k_2}{2k_1}\right) + \frac{a-k^2}{a}\left\{1 - \frac{7k^2}{6k_1} + \frac{2k_2}{3k_1{}^2}\right\}\right.$$

where a is the initial concentration of both reactants and h the hydroxyl ion concentration at the time t. Determining k_2 by a study of the monoester, it was possible to estimate k. The method has not been found satisfactory for later stages in the reaction.

Westheimer and his associates[39] use a graphical integration method and find results to within 5% upto 50% reaction. French and his associates[40] have developed an equation involving a parameter of the $\theta = \int_o^t A dt$ which requires evaluation for each value of t by graphical integration and which was found satisfactory in periodate oxidation of dextrins.

The most satisfactory approach, however, is that of Frost and Schwemer for the hydrolysis of esters of dibasic acids.[41] Equivalent amounts of the reactants rather than the same molar quantities are taken so that the second step becomes quite prominent.

Hence

$$A_o = 2B_o \qquad C = A\text{-}2B$$

and

$$\frac{dA}{dt} = (2k_2 - k_1)AB - k_2A^2$$

The solution is effected with the introduction of dimensionless variables $\alpha = (A/A_o)$, $\beta = (B/B_o)$, $r = B_o k_1 t$ and $K = (k_2/k_1)$ and solving for α and r. The integral in the solution for r is evaluated for ratios of integers representing K. The method has been extended by McMillan for different initial concentrations[41a] and by Svirbeley[42] for other ratios of K as also for tribasic acid systems.[43]

In the system of dibasic esters one of the points of interest is the nature of the second stage of the reaction which involves a negatively charged anion and as such an ionic strength effect may be expected. Combining the Debye-Huckel definition of ion activity in relation to ionic strength and Bronsted's equation connecting K and K_o, we can get

$$ln\, K = ln\, K_o + 2Z_A Z_B \alpha\sqrt{\mu}$$

using a mean value for the distance of closest approach. The assumption has to be made that the transition state has the equilibrium ion atmosphere in spite of its short life. It is worthwhile examining available results to see how far we judge on the nature of the second stage as well as the accuracy of the Frost-Schwemer approach. The results are presented in Tables IX and X below.

TABLE IX

Alkaline Hydrolysis of Dibasic Esters
Ratio k_1/k_2

Ester	Water at 20°C Ingold et. al. (38)	70% Dioxan Svirbeley et. al (42) at 30°C	60% Dioxan at 40°C S.V.A. et. al (23)	80% Alcohol at 40°C Westheimer et. al (39)
Ethyl Malonate	91·38	118* 4·8†		178
Ethyl Succinate	9·62	2·61	4·732	
Ethyl glutarate	6·40		3·572‡	
Ethyl Adipate	5·00		3·43‡	4·78
Methyl Pimelate	4·34			
Methyl Suberate	3·91			
Methyl Azelate	3·61			
Ethyl Sebacate		1·92	2·025‡	
„ O-Phthalate			5·031	
Benzyl Succinate			5·057	
Methyl Succinate	9·66			

* in 50% Dioxan
† in 80% dioxan
‡ Preliminary unpublished data of Mr. R. V. Venkataratnam.

TABLE X

60% Dioxan solutions at 40°C

Ester	k_1	k_2	E E.Cal	E K. Cal	log PZ	log PZ
Ethyl succinate	0·1950	0·04120	10·12	10·82	6·3513	6·1682
Benzyl succinate	0·2640	0·05220	9·56	10·26	6·0919	5·8782
Ethyl o-phthalate	0·0239	0·00475	13·11	13·68	7·5286	7·1671
Ethyl glutarate	0·1614	0·04519	9·645	11·910	5·94	6·97
Ethyl adipate	0·1124	0·03276	9·847	11·910	6·70	8·99
Ethyl sebacate	·04338	0·02339	9·434	12·940	5·26	7·40

Because of the variations in the solvents used, temperature and concentrations, the results of different workers are not comparable but the general trend in the homologous series is none the less useful for study. The abnormal value reported by Westheimer for ethyl malonate in 80% ethanol is presumably because

the second stage is slowed down by reversible interaction with the solvent itself. While Ingold's observations show no difference in the ratios of the two rate constants, replacement of the small alkyl by benzyl causes a larger change. The rate constants and their ratios have been extensively used in the interpretation of the mechanism of the reactions in the second stage. In our view this is of doubtful validity as may be seen in the drift in values of this ratio with temperature and with solvent (vide Table XI).

TABLE XI

Variations of k_1/k_2 with temperature
(60% Dioxan solution)

Ester	30°C	35°C	40°C
Dibenzyl succinate ..	5·248	5·148	5·057
Diethyl succinate ..	4·914	4·800	4·732
Diethyl o-phthalate ..	5·178	5·150	5·140

The drift can arise from differences in either or both Arrhenius parameters and we find that the two show a correlation of the type postulated by Fairclough and Hinshelwood.[44] It is generally noticed that the activation energy is consistently higher for the second stage while the pre-exponential term consistently lower. The increased activation energy is only to be expected from the deactivating influence of the carboxyl ion but the smallness of the difference in activation energy shows that this is not enough to suggest any difference in mechanism. The changes in entropy of activation are, however, of larger magnitude showing the importance of orientation in the transition state topology. From the mechanistic viewpoint, the trend in the ratios of the first and second stages gives an indication of the effect of separation of the carboxyl ion from the carbethoxy group and the ratio tends to a limiting value.

It should be mentioned that our values for the second stage of reactions agree within the limits of experimental error with the values obtained from a study of the half ester.

It is a feature of the mechanism indicated earlier that the transition state, being more polar than the initial reactant ester, should be solvated to a greater degree. This necessarily implies a definite

arrangement of solvent molecules with a consequent reduction of the pre-exponential term of the rate equation. As Ogg has pointed out[45] the observed correlation between the two parameters is itself an indication of this. One cannot, however, conclude a priori that solvation has no influence on the activation energy. As Ingold had pointed out (loc cit) while energy changes dominate the rate, the more polar solvent molecules accelerate or retard according to the nature of the transition state. We shall be considering this aspect more fully in analyzing solvent influences.

There has been difference of opinion as to whether the second stage in dicarboxylic ester hydrolysis is of the ionic or ion-dipole type.[46,47,48] Results reported for ionic strength effects are widely at variance. Most observations indicate that this is in any case small. Westheimer considers the second stage to be ionic[49] and Svirbeley has reported both negative and positive primary salt effects.[50] Hammett and Burnett,[51] consider that the second step is not seriously affected by ionic strength. Hoppe[52] has demonstrated the presence of specific salt effects to account for which he has postulated chelate intermediates in the transtition state. Our own observations indicate negligible ionic strength effects but to specific salt effects. No drift in rate is noticed for changes in ionic strength of 25% (Table XII)[53]

TABLE XII

Hydrolysis of sodium monoethyl succinate at 40°
(Concentration of reactants 0·005M)

Time	Mean ionic strength	Rate constant
60	0·010150	0·1036
190	0·010459	0·1068
460	0·010974	0·1053
540	0·011283	0·1063
880	0·011593	0·1063
1380	0·012109	0·1057
2090	0·012625	0·1057
Rate constant in the presence of KCl		0·1260

As a further test of whether the second stage is ion-ion or ion-dipole type, we can compute the probable dimensions of the transition state. Scatchard[54] has shown how this can be done for

the former type. Adopting Frost and Schwemer's method for evaluating rate constants and the topology of the transition state used by Laidler and Landskroener[55] values of molecular radii could be obtained for the reaction centre for the ion dipole reactions. These are given in Table XIII. The values clearly show that an ion-di-

TABLE XIII

Ester	Molecular radius by Scatchard's method	Molecular radius by Laidler's method
Diethyl succinate:		
Dioxan solution ..	76·88	2·90
Acetone solution ..	20·76	3·00
Dibenzyl succinate:		
Dioxan solution ..	134·20	2·85
Acetone solution ..	16·85	3·00
Diethyl phthalate:		
Dioxan solution ..	58·68	2·90
Acetone solution ..	13·31	3·20

pole mechanism is nearer the truth. No doubt, the half ester is a charged body but its influence is not much more than an external centre affecting the dipole undergoing the reaction. In the case of oxalate and malonates, the proximity may cause abnormal effects but available data is inadequate for assessment. The use of Lamer's method[56] for evaluating the distance of closest approach leads to unreal values. This absence of agreement between thermodynamic and collision calculations has been noticed by Svirbeley with ethyl malonate hydrolysis.[56]

Further work is necessary before we can fully elucidate all aspects of polybasic ester hydrolysis. It appears that a kinetic distinction between different conformations is possible judging from the trends of structural behaviour. Certain aspects of the problem are considered under solvent influences later. It is quite possible that the mechanism will have to be treated as a resultant effect of both intramolecular and environmental influences.

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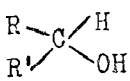
Lecture IV

KINETICS OF OXIDATION OF SECONDARY ALCOHOLS AND ALDEHYDES

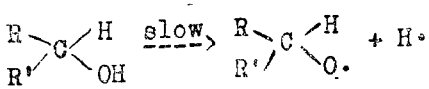
The oxidation of carbon compounds have been extensively studied using a variety of oxidizing agents and there are a number of reviews of the subject as well. As is the case with many such reactions, the mechanistic studies pose a number of problems. The position can be well illustrated with the various studies on the oxidations of secondary alcohols and of aldehydes by chromic acid.

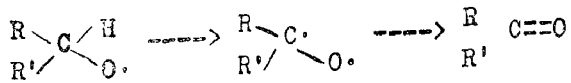
The oxidation mechanism can involve either a homolytic or a heterolytic fission in the rate determining stage according as we have a one electron or a two electron process at this stage. It is not necessary that the mechanism should be the same for the two groups of compounds though some similarity is not ruled out. Again one electron transfers imply a free radical intermediate with attendant chain processes for the types of compounds considered in our present study.

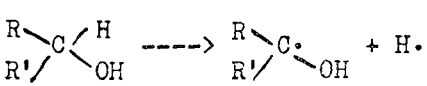
We can broadly divide the probable processes thus (Table I). The qualitative yield of ketones and the absence of glycols with

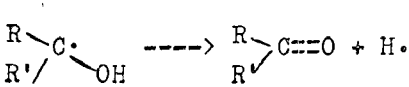
I. Oxidations of 

A. Homolytic fission

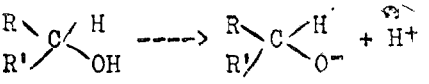
(i) 

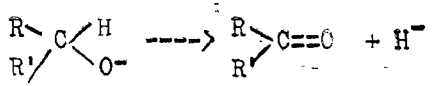


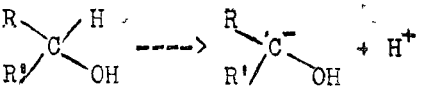
(ii) 

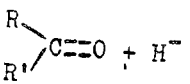


B. Heterolytic fission

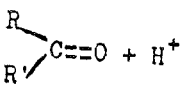
(i) 



(ii) 

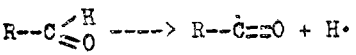


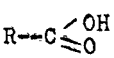
(iii) 



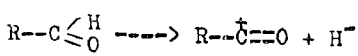
II. Oxidations of 

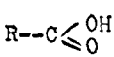
A. Homolytic fission



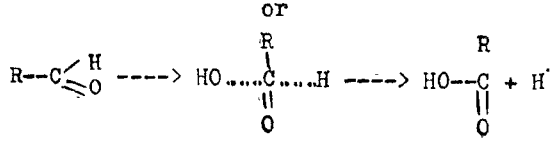


B. Heterolytic fission

(i) 



or

(ii) 

a tertiary carbon atom[1] preclude a homolytic process in the oxidation of secondary alcohols. Auto-oxidation of aldehydes proceeds essentially by a free radical mechanism. Mosher has shown that[2] the distinct difference in the rates of auto-oxidation and oxidation by chromic acid goes against a free radical process in the latter. We have then to choose between the two alternative heterolytic processes in both groups of compounds.

It will be convenient to consider the alcohols and the aldehydes separately first and then to compare the results. Westheimer and his associates[3] have clearly shown from their studies with the deuterated compounds that in the rate determining step, a C—H bond is broken in the secondary alcohol. The earlier work of Westheimer and Nicolaides[4] indicated such a situation, but this was clearly demonstrated by the later work of Westheimer and Cohen[5] who find that $D_3C\text{-}CHOH\text{-}CD_3$ oxidizes at the same rate as $H_3C\text{-}CHOH\text{-}CH_3$ but $H_3C\text{-}CDOH\text{-}CH_3$ is oxidized only at 1/7th of the rate under similar experimental condition. We shall see later how the same conclusion can be drawn without isotopic studies. The same conclusion was also reached by Kaplan using tritium as the indicator.[6] These observations rule out mechanisms which involve rupture of the O—H bond as rate determining in many cases. We have thus to choose between mechanisms B(ii) and B(iii).

Westheimer and his associates have preferred a mechanism involving the loss of hydrogen as a proton in the rate determining step and an essential feature of their mechanisms is the formation of an ester of chromic acid before oxidation takes place. The active agent is $HCrO^-_4$. The mechanism suggested is as follows:

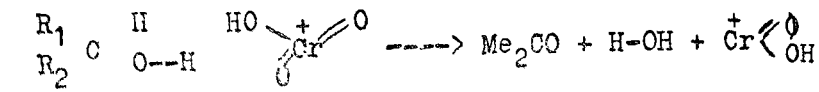
$$(HCrO_4)^- + 2H^+ + HO\text{--}\overset{R\ R}{\overset{|\ \ |}{C}}\text{--}H \xrightleftharpoons{fast} H\text{--}\overset{R\ R}{\overset{|\ \ |}{C}}\text{--}OCrO_2\overset{+}{O}H_2 + H_2O$$

$$H_2O: + H\text{--}\overset{R\ R}{\overset{|\ \ |}{C}}\text{--}O\text{--}CrO_2\text{--}OH_2 \xrightarrow{slow} H_3\overset{+}{O} + \overset{R}{\underset{R}{\diagdown\!\!\diagup}}C{=}O + H_2CrO_3$$

It will be noticed that, in the slow rate determining step, water is a reactant and where it is not present in large excess one may expect a reduction in the rate with decrease in water content. The reaction is, however, much faster in 86·5% acetic acid than in water.

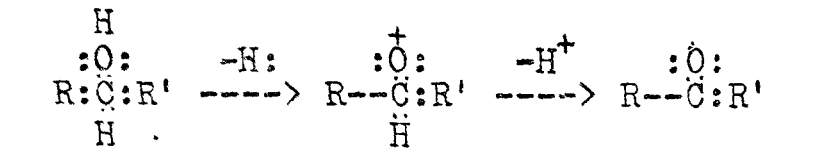
An alternative mechanism has been suggested by Rocek and his collaborators.[7,8] The approach is based on a generalization of

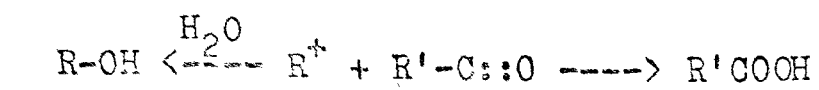
Zucker and Hammett[9] correlating participation of water in the transition state and the acidity functions H_0. They find that intervention of water is not essential as a proton abstractor and the formation of the ester itself is not a prerequisite. The mechanism suggested is essentially a hydride ion transfer:



The Westheimer mechanism in which water functions as a base requires base catalysis but the reported results are conflicting where pyridine has been used as a catalyst.

It is necessary to mention that there are structural conditions in which rupture of the O—H bond initially cannot be ruled out. Using alcohols with a tertiary alkyl group in the α position of the hydroxyl-bearing carbon, Mosher and Whitmore[10] obtained the corresponding tertiary alcohols during oxidation with chromic acid. They suggest the following scheme:





The position of rupture in the region of electron deficiency,[11] the nature of the neopentyl group,[12] and steric strains[13] have been brought into the picture. Westheimer[14] brings this cleavage also into his scheme based on chromic ester formation while Waters[15] accounts for the observation by a free radical mechanism. The system is obviously complex and more work is called for before one can decide on the mechanism.

Alcohol systems where cleavage is absent can, however, indicate the conditions for testing the alternative mechanisms. Since the C—H bond is involved in the rate determining step, the polarity of the bond may be expected to give a clue. Coulson's treatment of the methane molecule[16] has been extended by Walsh whose analysis shows a polarity $\overset{+}{\text{C}}\text{—}\overset{-}{\text{H}}$ in aliphatic compounds.[17] This has been further confirmed by the work of Hartman[18] and by

Gent.[19] The O—H bond shows a polarity in which hydrogen is the positive end of the dipole. A difference has then to be expected between structures where C—H bond rupture is involved and those where the O—H bond is involved. In the systems studied, one can then expect that structural conditions favour a hydride ion removal.

If hydrogen is lost as the anion, the residual carbon atom becomes a carbonium ion, with the valence electrons becoming sp^2 hybrids having a planar configuration.[20] The inherent instability of this charged body is partly neutralized by solvation and solvents which can effect this stabilization may be expected to favour a mechanism involving this structure. It is well known that in the aliphatic compounds secondary carbonium ions can have moderate stability.

Removal of hydrogen as a proton in the rate determining step indicates an electrophilic attack on the carbon atom. The large free energy of the proton makes the loss of a bare proton less likely than direct displacement of hydride ion and C—H bond rupture has then to be essentially a nucleophilic attack on hydrogen.[21] The transition state has then to behave as a strong acid which cannot be the case with the intermediate postulated by Westheimer.

We can now examine the reaction more closely taking both structural and environmental factors into account The results obtained in our laboratories under conditions in which the hydrogen ion activity was kept constant are presented in Table II, III and IV.[22]

TABLE II

Rate constants at 35°C and 50°C

Alcohol	$k_2 \times 10^5$ at 35°C	$k_2 \times 10^5$ at 50°C
Isopropyl alcohol ..	5 83	16·20
Butanol-2 ..	10·35	26·67
Pentanol-2 ..	14·97	35·67
Octanol-2 ..	11·89	31·59
α-Phenylethanol ..	33·13	89 13
Benzhydrol ..	62·00	166 63
Fluorenol ..	127·4	355·59

TABLE III

Oxidation of some aliphatic secondary alcohols by chromic acid in acetic acid solutions
Thermodynamic constants for the reaction

Compound	Activation energy K. Cal	$\triangle H^{\pm}$ K. Cal	$\triangle F^{\pm}$ (per mole) K. Cal	$\log_{10}PZ$	$\triangle S^{\pm}$ e.u.
Isopropyl alcohol ..	13·66	13·04	23·58	5·4569	—33·61
Butanol-2 ..	12·86	12·24	23·24	5·1378	—35·14
Pentanol-2 ..	12·56	11·94	23·08	5·0418	—35·58
Octanol-2 ..	13·11	12·49	23·16	5·375	—34·09
dl α-Phenylethanol ..	13·44	12·82	22·51	5·8467	—30·98
Benzhydrol ..	13·06	12·44	22·13	6·0525	—30·95
Fluorenol ..	13·99	12·77	21·68	6·6004	—28·47
Cyclopentanol ..	13·15	12·53	22·96	5·5322	—33·34
Cyclohexanol ..	13·47	12·85	23·16	5·6121	—32·97

TABLE IV

Influence of solvent on Activation energies

Solvent composition v/v Acetic Acid-water	E_a in K. Cals.						
	Propanol-2	Butanol-2	Pentanol-2	Octanol-2	Benzhydrol	Fluorenol	Cyclohexanol
30	15·82	14·89	14·69				
40	15·09	14·26	14·03				15·10
50	14·31	13·54	13·45		13·50	14·16	14·26
60	13·66	12·86	12·56	13·11	13·06	13·39	13·47
70	12·86	12·21	11·89	12·28	12·46	12·57	12·49
80				11·50	11·86	11·80	

It will be readily observed that merely using the rate constants will be wholly misleading, as the magnitudes vary considerably with temperature, but qualitative comparisons are possible taking a set of values at the same temperature. The aliphatic secondary alcohols differ in only one of the alkyl substituents. It is well known that these function by the inductive effect and the observed order

$$n\text{-}C_3H_7 > C_2H_5 > CH_3$$

is the reverse of what one should expect for removal of a proton in the rate determining step.

It will be recalled that esterification of the alcohol by chromic acid is an essential stage in the Westheimer scheme. The work of Smith and Olson[23] as well as that of Skrabal[24] and his associates clearly show that the alkyl group retards esterification and saponification in the order:

$$Me < C_2H_5 < (CH_3)_2CH < (C_2H_5)(CH_2)CH < (C_2H_5)_2CH$$

It is obvious that preformation of an ester cannot be a prerequisite for oxidation. Further confirmation is provided by the activation energy values which are invariably much lower than observed values of bimolecular esterification reactions. Ester formation however can be a competing reaction.

The thermodynamic constants provide us with a better criterion of comparison. Whether one takes the experimental activation energy or the heat and free energy of the reaction, there is a distinct lowering with the increased +I effect of substituents. The case of octanol-2 is interesting in that we notice a rise in activation energy. Apparently, the chain of six carbon atoms which can coil up such that the terminal groups oppose the general inductive effect with a consequent rise in activation energy. The size of groups also makes itself felt in a larger entropy of activation. The relative reactivities of cyclopentanol and cyclohexanol have to be linked up with the strain in ring systems on lines similar to those put forward by H. C. Brown and coworkers.[25] The ratio of the rate constant is in agreement with those of Kinvila and Becker[26] for the same temperature but actual values differ because of variation in experimental conditions. As with other reactions, the Fairclough-Hinshelwood comparison between the parameters can be made (Figure 1)

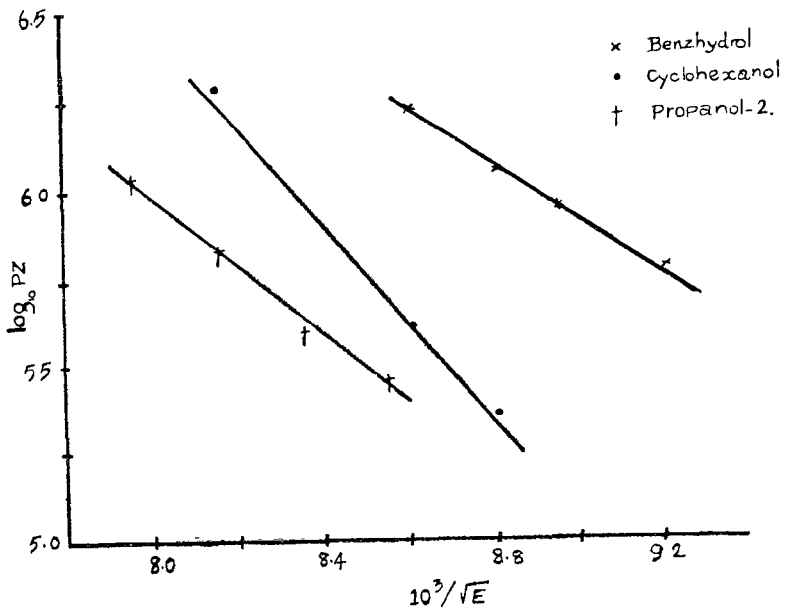


FIG 1

The linear relationship clearly indicates that both parameters are operative in the reaction and using the rate constant alone is inadequate for comparison. Even here there are limitations. Comparison has to be under constant environmental conditions. The small range in the activation energies in any single solvent system indicates an essentially similar process, the variation reflecting the difference in C—H bond energy.

We can next examine the nature of the reactants. Dielectric constant data are not available for the acetic acid-water system on account of experimental problems, of a conducting system. However, considering the chemical nature of the components, mean values may be computed by applying mixture laws, assuming a linear relationship in the dielectric constant. If attention is restricted to regions where the proportions are of comparable amounts, no serious error will be introduced. Following Laidler,[27] a linear relationship between $\log_{10}k$ and $D-1/(2D+1)$ should be obtained for a reaction between two dipolar molecules; While there should be a similar behaviour of $\log_{10}k$ and $1/D$ for an ion-dipole reaction. It will be seen from the figure (Figure 2) that the latter is true.

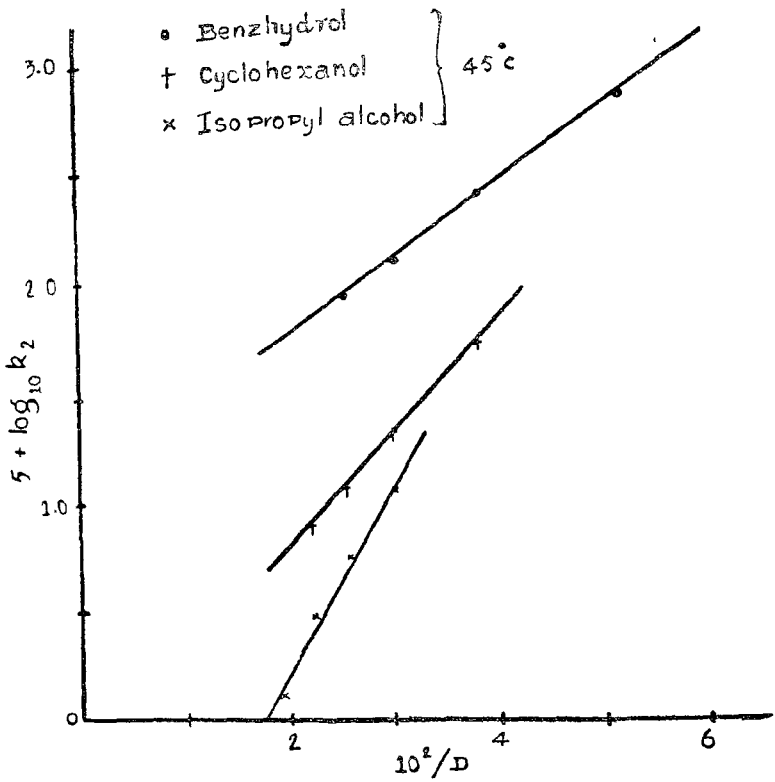


FIG 2

Postulating as ionic mechanism, we have to decide on the nature of the ionic species. Now Amis[28] has shown that in an ion-dipole reaction, the rate increases with dielectric constant if the ion is negatively charged and has an opposite trend if the ion is positively charged. The slope of the curves in figure (2) shows that the active species in the reaction is positively charged and may be of the type $(HCrO_3)^+$ or $(H_3CrO_4)^+$.

The postulate of this reactant clarifies many aspects of the oxidation reaction. The transition state has to be less polar than the initial state on account of the greater dispersal of charges. On the basis of the Born-Kirkwood equation, Hudson and Saville[29] have deduced the dispersal of charges for SN^2 reaction in the transition state and the same conclusions could be applied to E_2 type reactions also. This means that the solvation of the transition state is less than that of the initial reactants. The addition of a polar solvent then leads to an increased activation energy and a

slower reaction. This picture is confirmed by a close linear correlation between the mole-fraction of water and the reaction rate. This preferential solvation by the more polar solvent has been noticed also by Smith and Leffler[30] in ternary mixtures. Hinshelwood and Fairclough[31] had also observed that if the polarity of a solvent indicates its ability to solvate then PZ values increase with polarity as we find from Table V.

TABLE V

Compound	Mole fraction of water	E (K. Cal/mole)	$\log_{10}PZ$
	0·8824	15·82	6·0422
	0·8283	15·09	5·8278
Isopropyl alcohol	0·7630	14·31	5·5776
	0·6822	13·66	5·4569
	0·5801	12·86	5·2595
	0·7630	13·50	6·225
	0·6822	13·06	6·0525
Benzhydrol	0·5801	12·46	5·9561
	0·4458	11·86	5·7800

In the region of increased acetic acid concentration, one cannot rule out the presence of acetyl chromic acid or its conjugate acid $(CH_3COOCrO_3H_2)^+$ which are even more powerful oxidizing agents. From the general trend, our preference has to be for the charged species.

The next aspect of the mechanism is the determination of labile intermediates as the final product of reduction of chromic acid is tervalent chromium and the loss of three electrons cannot obviously be a single step. By the use of oxidizable ions which involve only a one electron transfer as competing agents, Westheimer[32] has been able to deduce the nature of the chromium species formed. Under our conditions of experiment, the oxidized manganese remained in solution and no quantitative significance could be attached to the reduced oxidation rate for the alcohol, but the use of Ce^{3+} confirmed a two-electron transfer in the rate determining step.

Before formulating a reasonable mechanism taking into account all the observations, reference should be made to the work of Kwart and Francis on oxidation of substituted phenyl ethyl alcohols.[33] Jaffé[34] fitted the rate constants into a Hammett relation and

found a value of $\rho = -1 \cdot 01$ in aqueous acetic acid. The rate constants are given in Table VI. The order is quite the reverse of what one can expect for proton removal in the rate determining step.

TABLE VI

Substituent	k_2 mole^{-1}/sec^{-1}	
p—OCH_3	2·13	
p—tC_4H_9	1·38	Solvent 30% HAc
p—CH_3	1·06	Temperature 30°C = 0·40
p—H	0·802	$H^+ = 0 \cdot 250$ M Perchloric Acid
p—Cl	9·612	
p—NO_2	0·160	

We can now suggest the following mechanism, as an alternative, for the process with reference to the chromium species:

A. $Cr(VI) + RCHOHR' \longrightarrow Cr(IV) + RCOR'$

$Cr(IV) + Cr(VI) \longrightarrow 2\ Cr(V)$

$Cr\ (V) + RCHOHR' \longrightarrow RCOR' + Cr(III)$

B. $Cr(VI) + RCHOHR' \longrightarrow Cr(IV) + RCOR'$

$2\ Cr\ (IV) \longrightarrow Cr(V) + Cr(III)$

$Cr(V) + RCHOHR' \longrightarrow RCOR' + Cr(III)$

Mechanism B is to be preferred as disproportionation of a partially oxidizing ion is a normal observation and the small retardation noticed by the initial addition of Cr(III ions is consistent with such a picture.

The mechanism of the reaction can then be visualized as a cyclic process of the type postulated by Rocek involving

(a) Hydride ion transfer from the C—H group of the alcohol system;

(b) the oxidizing species is a positively charged species $(HCrO_3)^+$ or $(CH_3COOCrO_3H_2)+$

The absence of any pronounced effect with pyridine can arise from two causes. Though hydride ion transfer can be base-catalyzed, this will be off-set by the formation of a stable complex between the labile chromium intermediate and pyridine. It is necessary to identify the transient species more definitely and a study of suitable complexing agents which do not otherwise interfere with the reaction together with simultaneous spectrophotometric studies may be expected to give the answer.

Turning to aldehydes, we are not in any satisfactory position on the mechanism. The fact that many aldehydes undergo auto-oxidation and reduce ammoniacal silver should not lead to the impression that aldehydes are easily oxidized. Aromatic aldehydes are not quantitatively oxidized by the latter reagent. One is constrained by the paucity of systematic rate studies with this group with a variety of oxidizing agents, favour a homolytic process; of compounds. Waters and his associates, analyzing oxidations with a variety of oxidizing agents, favour a homolytic process; chromic acid and similar compounds function as dehydrogenators abstracting atomic hydrogen.[35] According to Evans and Baxendale[36] there is "no evidence that two electrons are exchanged in a single step". However, Mosher and Preiss[37] report that while aliphatic aldehydes are not easily oxidized, a mixture of the alcohol and aldehyde undergoes oxidation faster than either and find that no free radical mechanism is possible.

The mechanism that is holding the field is the one put forward by Graham and Westheimer[38] and supported by Wiberg and Mill.[39] Ester formation with chromic acid is postulated as the initial step catalyzed by hydrogen ions, the suggested scheme being:

$$RCHO + H \longrightarrow R\overset{+}{C}HOH$$

$$R\underset{H}{\overset{OH}{C}}+ + HCrO_4^- \xrightarrow{-\ fast} R\underset{H}{\overset{OH}{C}}-O-CrO_2-OH$$

$$R-\overset{OH}{C}=O + H^+ + (CrO_2) + OH^-$$

Before examining the mechanism, it is worthwhile analyzing the experimental observation. Graham and Westheimer[40] find the reaction to be first order with reference to aldehydes as well as chromic acid, and shows a linear dependence on the Hammett acidity function. A comparison of C_6H_5CDO and C_6H_5CHO showed that as with secondary alcohols, the rate determining step involves the C—H bond and both manganous and cerous ions reduce the oxidation rate. These authors postulate further that dominant species in acetic and solution is $CH_3COOCrO^-_3$.

Wiberg and Mill[41] examine a number of substituted benzaldehydes. Their results are presented is Table VII. The oxidation of benzaldehyde indicates an activation energy of 13·2 K·clas and an entropy of activation of −28 units.

TABLE VII

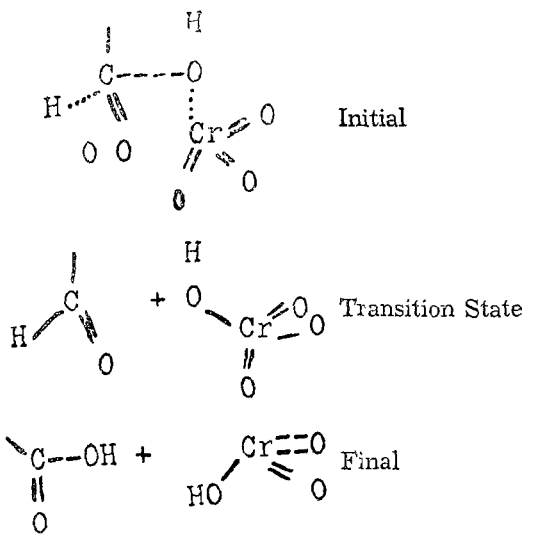
Substituent	$1\,k \times 10^3$ mol sec^{-1}
p—OMe	1·75
p—Me	4·26
p—H	6·17
p—Cl	8·50
p—NO_2	46·8
m—OMe	11·1
m—Cl	11·8
m—NO_2	29·6

By the use of O^{18} isotope in permanganate oxidations the postuate of an ester of aldehyde hydrate is put forward.

The choice of a common mechanism for permanganate and chromic acid mechanism and even the question of ester formation becomes suspect when one notices two opposite kinds of behaviour in the substituted aldehyde. In a recent review by Waters[42] we find under oxidation of aldehydes by chromic acid the statement "Electron attracting groups (e.g. NO_2) accelerate the oxidation as would be expected for the shift of the equilibrium of reaction in formation of cations $(ArCHOH)^+$ and the effects of substituents have magnitudes according with Hammett's function". In the same review under permanganate oxidations of aromatic aldehydes we find that "A Hammett plot can be drawn for relative oxidation rates of substituted aromatic aldehydes, showing that electron attracting groups, by preventing the formation of the organic cation, decrease the rate of the reaction."

As in the case of alcohol oxidation, the influence of substituent groups can be understood without the need to postulate ester formation or proton removal in the rate determining step. A difficulty in the postulated initial intermediate is the location of the charge on carbon. The charge can be equally on oxygen. We had noticed earlier that in the case of secondary alcohol oxidation, the active oxidizing agent is most probably a positively charged ion. We have to examine the possible entities for aldehyde oxidations. Structurally, the carbon atom in the aldehyde group is one having a trigonal configuration of the electron system. The product of oxidation is also one of the same category. The question naturally arises whether it is necessary to postulate an intermediate

with different bond hybridizations. The structure of the CrO_4 group is tetrachedral and whether we consider the ion-pair H^+ and $HCrO^-{}_4$ or a neutral molecule as the reagent, a mechanism S_N2 type can account for the observed trend without any change in the hybridization of the carbon:



If we thus require the C—H hydrogen to be removed as a hydride ion, it follows that electron attracting groups in the benzene nucleus of an aromatic aldehyde can facilities oxidation by location of positive charge on the carbonyl carbon. The relative influence of m-substituents and the difference exhibited by the corresponding para compounds further indicate removal of hydrogen as a hydride ion.

Unpublished observations of Jayaraman in 60% acetic acid solution at 70°C gave the following results with substituents relative to unsubstituted benzaldehyde for the rate constants:

$H-C_6H_4-CHO$	1
$p-Cl-C_6H_4-CHO$	1·6
$p-NO_2-C_6H_4-CHO$	3·9
$m-NO_2-C_6H_4-CHO$	2·2

The trend is similar to that reported by Wiberg and Mill[41] but absolute values differ on account of differing experimental conditions. It is difficult at the present stage to decide on the mechanism. If our tentative mechanism holds, C_6H_5CDO should show

loss of deuterium during oxidation and if chromic acid contains isotopic oxygen, O^{18}, the carboxylic acid should show the presence of this heavier oxygen. It is also necessary to obtain unequivocal evidence about the nature of the oxidizing agent. Our preliminary studies indicate that this is probably a neutral molecule or negatively charged one, thus differing from the alcohol oxidations. Further work is clearly called for.

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LECTURE V

INFLUENCE OF SOLVENTS ON REACTIONS

In the study of reaction mechanisms, the ideal conditions for comparison will be where data can be had for both gas phase and condensed phases. However, where the reaction involves ionic species no gas phase analogy is possible. Even in the case of the reaction between ethylene and bromine, the need for a polar environment has been noticed even for the gas phase.[1] In all reaction in solution some interactions of nearest neighbours will have a necessary part in reaction schemes and the importance of this will be quite pronounced in solvolytic reactions.

One of the problems of solution kinetics is the choice of a suitable physical model. Any molecular description of the rate process is related to a molecular description of solution behaviour. While a transition state description of the reacting system is possible, there is an unavoidable arbitrariness in defining the encounter between reactants as it depends on the forces between them and on the model of liquid state. It is possible to calculate the frequency of such encounters for an assumed lattice model, but experimental verification of any such formulae is precluded by limitations of activity coefficients and specific interactions.

In extending the transition state approach to reactions in solution, one has to reckon with solvation and solvation energy as well. It should be emphasized that in reactions in solution there is no general correlation between the empirical formula, reaction order and the molecularity of the mechanism. The transition state formation enables a reasonable picture of the process to be formed, but one has to assume a suitable topology for this stage consistent with reactants and products. Any medium which favours the association of reactants can be expected to facilitate a reaction. As such, any reaction which involves one or more polarized molecules or ions will take place more readily in a polar environment. From the theoretical point of view it will be of interest to discuss the influence of pressure on reactions as well, but in our present study we shall be dealing only with atmospheric pressure conditions.

Where charged species are involved, and solutions of moderate concentrations are considered, interaction between solvent species

and the ionic species can be sufficient to overcome inter-ionic forces and differences in solvation of reactants, transition state and products will not be negligible as a factor in determining the reaction rate. An estimate of the forces between univalent ions treated as point charges from simple theory leads to energy values of the same order of magnitude as are met with in chemical reactions, even after allowing for the effect of dielectric constant. The simplest approach to systems which involve only ionic species is the Debye-Huckel theory and has been successfully treated in the first instance by Bronsted[2] and by Bjerrum.[3] In spite of what is probably an oversimplification of the Debye-Huckel model, Bjerrum's proposal that any pair of ions whose interaction energy is of the order of $2k$T or more should be treated as an ion pair and not as independent ions is an important step in the right direction. It will be seen later that this complication has to be reckoned with, especially in solvents of low dielectric constant. In fact, where the dielectric constant approaches that of nonpolar liquids, one cannot exclude even triple ions and quadruplets.

In dealing with ion-dipole reactions, three distinct approaches are available. Kirkwood applies electrostatic theory to deduce the value of free energy of solvation and thence the activities of the polar molecules. Laidler and Eyring,[4] assuming a spherical activated complex, obtain an expression for a reaction ions of the form

$$\ln k = \ln k_o + \frac{\varepsilon^2}{2k\mathrm{T}}\left(\frac{1}{\mathrm{D}} - 1\right)\left[\frac{z_{\mathrm{A}}^2}{r_{\mathrm{A}}} + \frac{z_{\mathrm{B}}^2}{r_{\mathrm{B}}} - \frac{(z_{\mathrm{A}} + z_{\mathrm{B}})^2}{r_{\neq}}\right]$$

$$+ \frac{\varepsilon^2 z_{\mathrm{A}} z_{\mathrm{B}}}{\mathrm{D}k\mathrm{T}} \cdot \frac{k}{1 \mp ka} + \Sigma\varphi/k\mathrm{T}$$

The last term is a non electrostatic part and if one of the reactants is a neutral molecule $z_{\mathrm{B}}=0$, and if ln k is plotted against 1/D a straight line having the slope

$$\frac{d \ln k}{d(1/\mathrm{D})} = \frac{\varepsilon^2 z_{\mathrm{A}}^2}{2k\mathrm{T}}\left(\frac{1}{r} - \frac{1}{r_{\neq}}\right)$$

can be obtained. Since the activated complex is generally of larger dimensions than the ionic reactant, this equation implies that the

rate should decrease with increasing dielectric constant. Bronsted has shown that this equation can be reduced to the form

$$\log k = \log k_o^s + 1{\cdot}02\, z_A z_B \sqrt{\mu}$$

if the dielectric constant and temperature are constant. As before, if $z_B=0$, this equation of Bronsted shows that the reaction should be independent of ionic sterength.

The generalization indicated are subject to limitations arising from the assumption on which the equations have been deduced. The solvent is treated as a homogeneous medium of uniform dielectric constant. Especially in mixed solvents this is of doubtful validity. Water molecules may be preferentially oriented and, in the vicinity of the ion, the dielectric constant may be different from that of the bulk. Where dipolar molecules are involved, the assumption that $z_B=0$ is also quite incorrect. All that can be said is that in the region of solvent mixtures where the extreme compositions are not involved, the expected linear relationship will hold good.

A slightly different approach has been made by Amis and Jaffé.[6] Following the procedure of Christiansen[7] and of Scatchard[8] and using the dependence of potential on both r and θ for a reaction between an ion and a dipole of moment the rate constants are found to be connected by the equation:

$$ln\, k = ln\, k_{K=0} + \frac{\varepsilon z_A \cos\theta_o}{DkTr_o^2}\left(\mu_o^* - \frac{\mu^*(1+kr_o)}{e^{kr_o}}\right)$$

The relative magnitudes of the quantities in the brackets is such that for positive ionic reactants, k should increase with decreasing rate as ionic strength increases and similarly, the rate should increase with decreasing dielectric constant. Similarly, if the ionic component is negative, the reverse effects should be noticed. Moelwyn-Hughes[9] has deduced a still further variant for this type of reaction. This is the simplest approach. Neglecting the polarization of the species the coulombic energy of the transition complex from the reactants of charge $z\varepsilon$ and dipole μ_B can be calculated for

different orientations. The interaction is a minimum for $\theta = O$. The influence of solvent is then finally obtained in the form:

$$ln\left(\frac{k}{k_o}\right)_{K\to O} = -\frac{z\ \varepsilon\mu}{Dr^2kT}\cos\theta$$

This can have positive or negative values depending on θ. If $\theta > 90$ increasing D will slow the reaction if the interaction is attractive while the reverse is the case for repulsive interactions. The equation has actually to be modified, to allow for the fact that the free energy of formation of the transition complex is of the same order of magnitude as the solvation energy. The result is that the influence of dielectric constant is not separable from the influence of the dipole moment and distance of closest approach of the solvent.

The complex nature of any assumed model precludes more than a qualitative picture, but none the less useful comparisons are possible and an identification of the reacting species can be effected by a study of solvent influence.

We now can examine how far some of the reaction we have studied can be fitted into the general picture. The alkaline hydrolysis of esters is an ion-dipole reaction in which one of the reactants is the negatively charged OH^-. The results of our studies in different solvent mixtures are presented in Tables I, II, III and IV.

The results clearly indicate the inadequacy of any interpretation of solvent effects on the basis of dielectric constants alone The initial addition of organic solvent affects the reaction rate in a direction opposite to what one expects from the Kirkwood-Eyring or Amis-Jaffe picture. For the same dielectric constant, the rates as well as the parameters of the Arrhenius rate equation show variations such that specific solvent effects will have to be analyzed. The behaviour in glycerol-water system is particularly interesting. No dielectric constant data are available for this system and because of the non-ideal behaviour of the mixtures, no useful information can be had by any linear computation. It is well known that all physical properties show the maximum deviation from ideal behaviour in 60% glycerol and in every ester studied in Table III, the minimum values of activation energy and pre-exponential term and the maximum rate are noticed.[15] The high viscosity of this system indicates the importance of the Frank-Rabinowitch cage effect in such reactions. Apart from the specific solvent effects in which the solvation of the proton is significant, in solvolytic reac-

TABLE I

Alkaline Hydrolysis of Ethyl Acetate in Solvent Mixtures of organic solvent and water (10, 11)

Vol % of organic component	Dioxan-water				Acetone-water			
	Dielectric constant (at 30°C)	k_2	constant Dielectric (at 40°C)	k_2	Dielectric constant (at 30°C)	k_2	Dielectric constant (at 40°C)	k_2
0	76·8	0·1619	73·12	0·2815	76·8	0·1619	73·12	0·2815
2		0·1649		0·2882		0·1628		0·2891
4		0·1682		0·2944		0·1657		0·2967
6		0·1699		0·3030		0·1674		0·3059
8		0·1735		0·3079		0·1645		0·3094
10	67·76	0·1669	64·49	0·2848	72·31	0·1589	68·95	0·2890
20	58·80	0·1279	56·84	0·2617	67·75	0·1399	64·60	0·2402
30	50·43	0·1122	47·05	0·2210	62·70	0·1151	59·80	0·1950
40	41·80	0·1007	39·54	0·1872	57·60	0·0979	54·99	0·1647
50	33·51	0·0858	31·66	0·1601	51·98	0·0795	49·62	0·1327
60	25·53	0·0743	24·11	0·1361				
70					39·53	0·0648	37·69	0·1037

TABLE II

Arrhenius parameters for the hydrolysis of Ethyl Acetate in acetone-water and dioxan-water systems

Vol % of organic solvent	Dioxan-water				Acetone-water			
	Acid Hydrolysis		Alkaline Hydrolysis		Acid Hydrolysis		Alkaline Hydrolysis	
	E Kcals	$\log_{10}PZ$	E Kcals	$\log_{10}PZ$	E Kcals	$\log_{10}PZ$	E Kcals	$\log_{10}PZ$
0	16·00	7·77	10·53	6·69	16·00	7·77	10·53	6·70
2			10·60	6·80			10·88	7·01
4			10·62	6·83			11·05	7·14
6			10·97	7·09			11·43	7·41
8			10·88	7·03			11·97	7·81
10	16·40	8·05	10·14	6·49	15·70	7·55	11·34	7·33
20	16·10	7·86	13·48	8·83	15·60	7·43	11·80	7·70
30			12·69	8·21			9·93	6·22
40	16·00	7·71	12·19	7·79	15·30	7·14	9·79	6·06
50			11·74	7·41			9·95	5·86
60	16·30	7·78	11·39	7·09	16·40	7·77		
70							8·84	5·19
80	17·20	8·23			16·80	7·87		
90	17·20	8·27			17·20	8·18		

TABLE III

Acid Hydrolysis of some aliphatic esters in glycerol-water systems

% glycerol	Ethyl Acetate			(12) Ethyl Propionate			Ethyl Chloracetate		
	$k_1 \times 10^3$ at 35°C	E Kcals	$\log_{10}PZ$	$k_1 \times 10^3$ at 35°C	E Kcals	$\log_{10}PZ$	$k_1 \times 10^3$ at 35°C	E Kcals	$\log_{10}PZ$
20	0·2557	16·62	8·238	0·2631	15·88	7·724	0·1782	15·16	7·041
40	0·2945	15·87	7·756	0·3080	15·25	7·301	0·2053	15·79	7·568
60	0·3226	14·37	6·785	0·3010	13·90	6·477	0·2316	14·65	6·832
80	0·2581	17·10	8·580	0·2509	16·13	7·869	0·2316	15·31	7·255

TABLE IV

Alkaline hydrolysis of esters of some Aromatic acids and of dibasic acids

(11)

Mole fraction of water	Dioxan-water							
	41·00		32·50		23·90		16·00	
Ester — Mole fraction of water	0·8789		0·8277		0·7506		0·6730	
	E	$\log_{10}$PZ	E	$\log_{10}$PZ	E	$\log_{10}$PZ	E	$\log_{10}$PZ
Ethyl benzoate	12·22	6·8000	12·76	7·3000	13·23	7·6000	13·95	7·7600
Benzyl benzoate			12·47	7·0663	12·790	7·2201	13·28	7·5181
p-nitro Ethyl benzoate					11·57	8·1019	12·28	8·5528
Ethyl cinnamate			12·53	6·7892	13·22	7·5961	13·71	7·8930
Benzyl cinnamate			11·96	6·9972	12·53	7·3356	13·12	7·6793
Diethyl succinate (I Step			9·75	6·1125	10·12	6·3513	10·49	6·5424
(II Step)			10·28	5·8098	10·82	6·1682	11·27	6·4696
Dibenzyl succinate (I Step)			9·226	5·9304	9·557	6·0919	9·9650	6·2716
(II Step)			9·997	5·7039	10·26	5·8782	10·70	6·1799

(Continued overleaf)

TABLE IV

Alkaline hydrolysis of esters of some Aromatic acids and of dibasic acids
(Continued from previous page)

Dielectric constant at 30 C		Acetone-water					
		53·00		49·00		44·00	
Ester	Dielectric constant at 30 C	0·8058		0·7341		0·6393	
		E	$\log_{10}$PZ	E	$\log_{10}$PZ	E	$\log_{10}$PZ
Ethyl Benzoate Benzyl Benzoate		13·55	7·7371	13·96	7·9643	14·53	8·2495
Ethyl cinnamate		14·04	8·2089	14·44	8·3639	15·04	8·7311
Benzyl cinnamate		13·61	8·2626	14·20	8·4208	14·58	8·5669
Diethyl succinate I Step		10·54	6·6703	10·81	6·7742	11·02	6·8203
II Step		11·04	6·2481	11·24	6·3792	11·70	6·6987
Dibenzyl succinate I Step		10·01	6·3720	10·31	6·4953	10·68	6·6164
II Step		10·30	5·8129	10·81	6·1556	11·00	6·2674

tions, some correlation between the mole fraction of water and the the rate may be expected. This will be noticed to be the case from an examination of the data in Table II. We have actually found a linear relationship.[11,16] Further, for small addition of one solvent to another, the structure breaking effect of such addition can lead to abnormal values in entropy of activation as we have observed with both dioxan-water and acetone-water systems.

Where alternative mechanisms are possible, these may not be influenced in the same manner by the solvent environment. While no positive proof is possible, it is reasonable to conclude that where the effect is the same, the same mechanism is probable. On this basis, we can infer that all the esters considered in Table IV hydrolyze by the mechanism-viz., acyl-oxygen fission and the mechanism is the same for both first and second stages in the hydrolysis of esters of dibasic acids. The results clearly show that since the first stage is of the ion-dipole type, the second is also essentially an ion-dipole reaction, and not one between ions of charges of the same sign.

The analysis of solvent influence on other ion-dipole reactions, however, may be expected to reveal features that can help in testing postulated reaction mechanisms. Oxidation of secondary alcohols by chromic acid can be taken as a typical example of this. Westheimer's mechanism postulates an initial formation of a chromic ester with possibly an intramolecular proton transfer.[17] The observation that the reaction between chromic acid and alcohol is faster in 86·5% acetic acid than in water is attributed to a better oxidizing agent in acetyl chromic acid.[18,19,20] A further feature of the mechanism is the participation of water as a base. It has been observed by us that consistently the activation energy for the reaction is lowered with the lowering of the water content (Table V)[21].

Acetic acid-water systems present difficulties in the way of dielectric constants, and no data are available. However, assuming a linear relationship between the composition and dielectric constant, the approximate values given in the table could be deduced. While the structural influence on activation energy is small, there are large variations with solvent composition and the pre-exponential factors are well separated. The result is a spread in the rate constants. Applying the criterion for the nature of the reacting species indicated by Amis,[23] the slope of the log k—$\frac{1}{D}$ line indi-

TABLE V

Dielectric constant at 45 C	E for alcohol oxidized						Mole fraction water
	Propanol-2	Butanol-2	Pentanol-2	Benzhydrol	Fluorenol	Cyclohexanol	
52·2	15·82	14·89	14·69				0·8824
45·5	15·09	14·26	14·03			15·20	0·8283
39·0	14·31	13·54	13·45	13·50	14·16	14·26	0·7630
32·4	13·66	12·86	12·45	13·06	13·39	13·47	0·6822
26·05	12·86	12·21	11·89	12·46	12·57	12·49	0·5801
19·45				11·86	11·80		0·4458

cates that we are having an ion-dipole reaction in which the ion carries a positive charge. The possibility of such a cationic species as $(\overset{+}{H}CrO_3)^+$ or $(H_3\overset{+}{C}rO_4)^+$ has been anticipated by Levitt[24] and more recently shown by Rocek.[25]

Now, the reaction between such an ion and the neutral molecule means that in the transition state, there will be a greater dispersal of charge with the result that the transition state will be less solvated than the reactants. A decrease in the polar environment should then favour the reaction. This is actually found to be the case. As a corollary, we have to expect the observed linear between the mole fraction of water and the activation energy, with a negative slope (Table V).

Another aspect of the solvent influence illustrated by this reaction is the electrophilic or nucleophilic behaviour. Our analysis of the reaction as a hydride ion transfer in the rate-determining step implies a transient carbonium ion formation. The life time of a carbonium ion is increased in solvents of nucleophilic type. If we take the acetic-acid-water system, the greater the acetic acid proportion the greater will be its nucleophilic behaviour. Hence a reaction involving this characteristic should be facilitated.[26]

We thus find the influence of solvent a convenient support to modify an accepted mechanism. Turning to another reaction which

TABLE VI

Reaction between Pyridine and Methyl iodide (28, 29)

Solvent	Dielectric constant	$k \times 10^5$	E	$\log_{10}PZ$
	21·45*			
Acetone	19·38**	58·43	11·28	4·70
60% alcohol-water	40		18·20	9·05
95% alcohol-water	27	8·367	17·82	8 41
100% alcohol	25*		18·00	8·32
78·3% alcohol-benzene			17·20	7·86
47·5% alcohol-benzene			15·90	6·99
15·5% alcohol-benzene			14·90	6·28
100% benzene	2·29$^\pi$		14·40	5·55

* at 20°C; ** at 40°C; π at 15°C.

has played a significant part in the history of the role of solvent on the reactions, we are confronted with other complications. The Menschutkin reaction[27] has been extensively studied and Menschutkin's data widely quoted. The difficulty in using these arises from the formation of precipitates in nonpolar solvents. Attention was drawn to this aspect by Edwards and Davies and Cox restricted their observations to homogenous conditions. The use of solvent mixtures is particularly interesting here. Confining attention to the reaction between pyridine and methyl iodide and between pyridine and benzyl bromide, we notice results which bring out many significant features in the reaction. These are presented in Table VI and VII.

TABLE VI-A

Solvent	log k×10⁵	E	D
Mesitylene ..	1·94	14·9	..
Toluene ..	2·07	14·50	2·37
Benzene ..	2·15	14·10	2·27
Chlorobenzene ..	2·60	13·90	5·60
Bromobenzene ..	2·70	13·70	
Iodobenzene ..	3·06	13·80	
Nitrobenzene ..	3·57	13·70	
Benzonitrile ..	3·50	13·70	
Anisole ..	2·83	13·20	

TABLE VII

Reaction between Pyridine and Benzyl Bromide in Benzene-alcohol system (30, 31)

Solvent	E	$\log_{10}$PZ
100% alcohol ..	14·36	8·63
90% „ ..	14·17	8·47
75% „ ..	14·18	8·43
50% „ ..	14·11	8·27
25% „ ..	16·06	9·438
10% „ ..	16·25	9·267
100% Benzene ..	13·15	6·394

In both cases the reaction may involve the following stages:

$$\left.\begin{array}{l} R-X \xrightarrow{\text{slow}} R^+ + X^- \\ \rightarrow N: + R^+ \xrightarrow{\text{fast}} \rightarrow \overset{+}{N}-R \end{array}\right\} \text{ I}$$

$$\left.\begin{array}{ll} \rightarrow N: + R-X & \text{Initial State} \\ \rightarrow N.....R.....X & \text{Transition State} \\ \rightarrow NR + X^- & \text{Final State} \end{array}\right\} \text{ II}$$

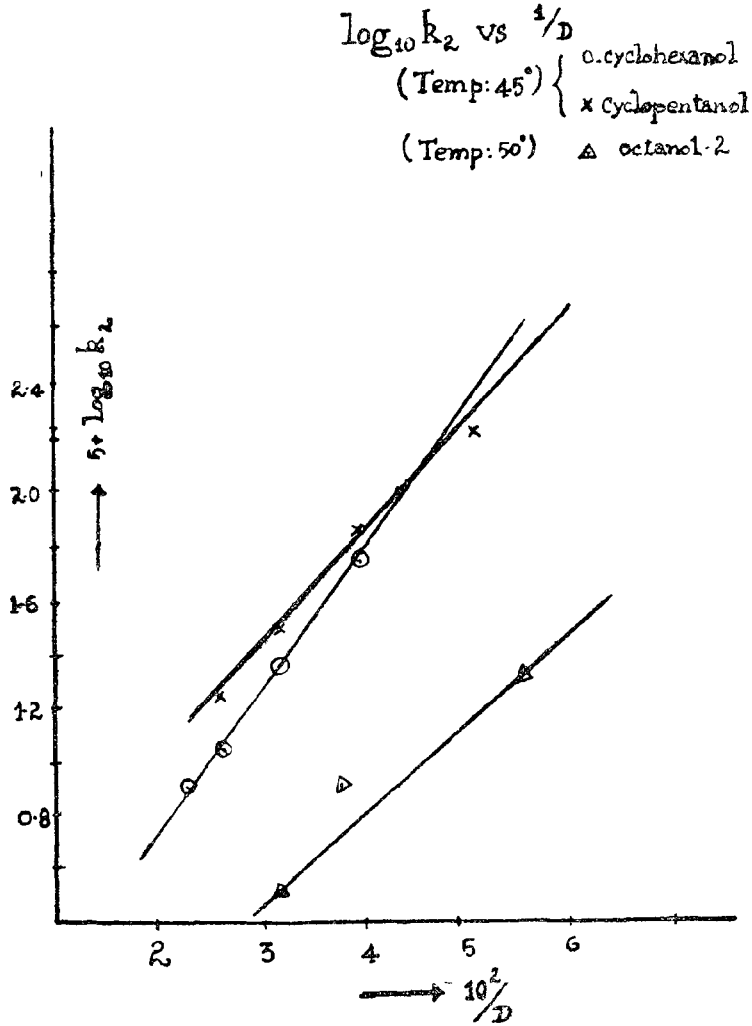

Fig 1

The consistent bimolecular rate constants rule out the first alternative in the present examples. The mechanism then requires a transition state which is nearer the products than the reactants and so should be more polar than the latter. The result is that one has to expect the transition state to be stabilized by solvation with a polar solvent. While we thus expect a polar environment to favour the course of the reaction, it is significant that in the benzene alcohol mixtures, it is the more polar environment that requires a larger activation energy for the reaction. In some solvolytic studies with alkyl halides using media of low dielectric constant, Hughes, Ingold and coworkers[33] suggest the formation of a quadrupole intermediate. In the present reaction a similar situation is not ruled out. For a dipole-dipole reaction we have to expect a linear correlation between $\frac{D-1}{2D+1}$ and log k, we find significant deviations.[32] As with ion dipole reactions we have thus to reckon with specific solvent effects here. Pyridine can form a hydrogen bonded complex with water or alcohol and the incipient positive charge apparently results in an added repulsion term for the interaction with a polarized alkyl halide. The absence of such a complication then

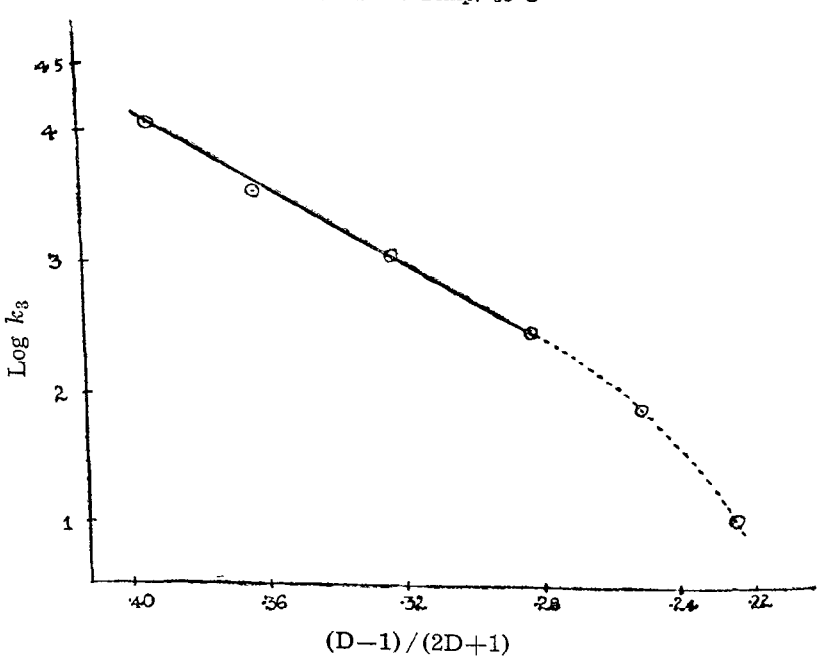

Fig 2a

accounts for a lowering of the activation energy in acetone and in nitrobenzene[31] compared to the alcohol systems. At the same time, one cannot also overlook the possibility of some contribution by mechanism I or solvolysis of the alkyl halide where water or alcohol are present with other alkyl halides. Any result in solvents where there is separation of the ionic product cannot also be of use in isolating solvent influence.

In the formation of quaternary ammonium compound, the nature, of the product restricts the choice of the experimental conditions for a homogenous reaction, but this may not be the case where the product is also only a polar molecule and not an ionic one. The example of olefine-bromine reaction is one such. We have noticed earlier[36,37] that structural considerations lead to a picture of concurrent bimolecular and termolecular mechanisms. It is interesting to examine the influence of a solvent.

Williams and James[34] observed that the dark reaction exhibited autocatalysis in carbon tetrachloride and our own studies in methylene chloride[35] showed a similar behaviour though the induction period was small. The difference arises from the greater polarity of methylene chloride. Earlier studies had also indicated the need for a polar environment for this reaction. The study of a series of substituted olefines indicated a fairly complex situation in which it was expected that solvent systems could give some clue. These studies showed that the reaction in carbon tetrachloride was essentially heterogeneous.[36] The reaction in glacial acetic acid was invariably homogeneous and since acetic acid and carbon tetrahloride are misible, the mixtures may be expected to give some information. It turns out that even compounds which show a dominant bimolecular mechanism in the pure acetic acid, the lowering of the dielectric constant tends to favour a termolecular mechanism. In between we have simultaneously both mechanisms. The trend of the rate constants in the solvent mixtures, however, shows clearly that the reaction is one between two polarized molecules in the rate determining step. The role of solvent becomes significant in the stabilization of the transition state. This activated complex is more polar than the reactants and solvation will be associated with the charge dispersal in the cationic resonance hybrid (cf. mechanism of Bromine addition [Lecture 2].[37, 38] It is also possible that some solvents may facilitate the reaction by facilitating the activation of Bromine. Under such conditions, using a solvent like Dioxan where this may be expected, it is possible that the bimolecular mechanism may be favoured. The significant contribution by the solvent environment can be readily seen from the data presented in Table VIII.

TABLE VIII

Reaction of bromine with olefines in Acetic Acid-CCl_4 mixtures

% Acetic acid	Dielectric constant	Allyl Chloride M/80			Stilbene M_3200			Dimethylacrylic acid		
		$\log_{10}k_3$	E	$\log_{10}PZ$	$\log_{10}k_3$	E	$\log_{10}PZ$	$\log_{10}k_3$	E	$\log_{10}PZ$
100	6·24	2·6593	2·82	2·94	4·0440			1·4237		
90	5 45	2·4368								
80	4 85	2·1380	1·07	1·14	3·5681	5·43	5·63	1 0795		
60	3·725	1·5412	1·08	0·55	3·0311	5·52	5·16	0·5670		
40	2·968				2·4840	5·34	4·49	1·9756		
20	2·514							1·1976		

In all the cases while a linear relationship of the Kirkwood form[39] is obtained in the region of higher dielectric constant, pronounced deviations are noticed in the regions of higher carbon tetrachloride content (Figure 2). These deviations can arise from changes in the solvation of the transition state as also the difference in dielectric constant of the reaction zone from that of the bulk value. This difference can arise from the segregation of the more polar component in the solvent mixture. A further difficulty in systems containing acetic acid is a possible increase in dielectric constant with temperature though an increase in concentration of the monomeric form.

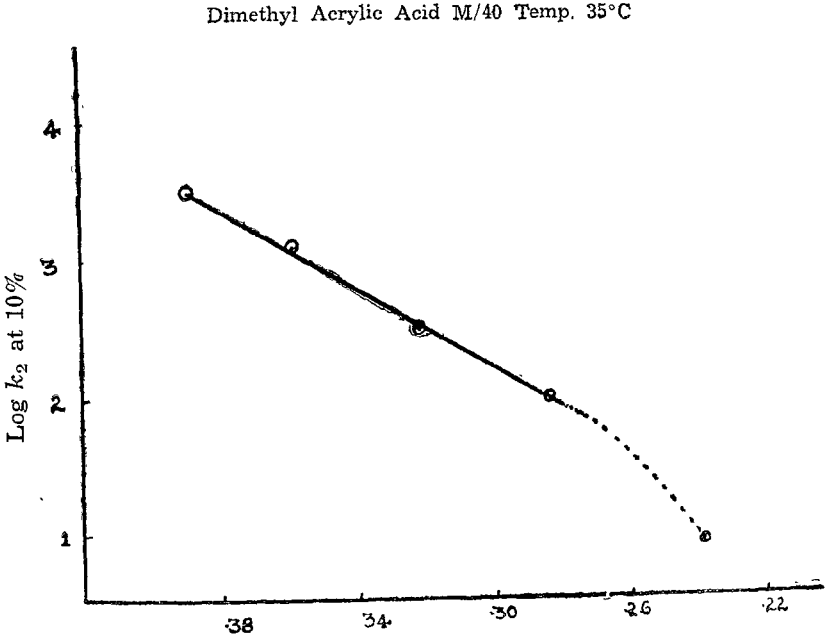

FIG 2b

Before concluding our analysis of solvent influences on the two types of reactions we have studied reference may be made to some interesting observations of Swain[40] and his associates on some decarboxylation reactions. His data on the decomposition of $CH_3(CH_2)_2COCH_2COOH$ are given in Table IX. Where data are provided for two temperatures estimated Arrhenius activation energies show very little difference from solvent to solvent while the rate data indicates an abnormal behaviour in 50% acetone.

TABLE IX

Solvent	$k_1 \times 10^4$
Water ..	8·5
18% acetone ..	9·0
50% „ ..	12·8
68% „ ..	8 3
91% „ ..	4·0
Benzene ..	1·35
Hexane ..	0·95

The concerted mechanism for transfer of hydrogen indicates a large change in entropy. The wide dispersion of rate with close values for activation energy is consistent with this behaviour. However, it is clear that further work is needed for more definite conclusion of the mechanism.

In examining the influence of a solvent on a reaction we thus find the need for a good deal of caution. The nature of the reaction may largely be correlated to the dielectric constant of the medium. Abnormalities and deviations arise through one or more of the following causes:

(1) Macroscopic dielectric constant differs from that of the reaction zone. This can be the case even in single solvents.
(2) Small additions of a second solvent has a structure breaking effect which is noticed in abnormal values of entropy changes.
(3) The solvent or one of the solvents may be a participant in the rate determining or other significant stage of reaction.
(4) In condensed media, especially with viscous liqiuids, the cage effect will not be negligible.
(5) Preferential solvation of the transition state can cause segregation of a more polar component in the reaction zone. Where non-hydroxylic solvents are involved ion pairing has also to be reckoned with.[41]
(6) Mechanistic changes can be brought about by the solvent environment.

Solvent influences can, however, be used to supplement the information provided by other studies so as to infer the probable mechanism of a reaction.

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A Note on Terminology and Classification of Joints in Sedimentary Rocks

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ABSTRACT

Existing terminology and classifications of joints in sedimentary rocks are briefly reviewed. A new approach is suggested in evolving a classification that takes into account all information available on a set of joints under study.

Introduction :

The term 'Joint' is said to have originated in the British coal fields where miners thought that rocks were jointed along fractures, just as bricks are put together in a wall.

Joints may be defined as divisional planes or surfaces that divide rocks, along which there has been no visible movement parallel to the plane or surface. Usually, though not necessarily, they are plane surfaces. Some times they are curved or warped. These openings may be continuous for long distances or may die out even within a few feet. Though joints are universal, they are best developed in fine grained deposits of homogenous composition.

Willis, B. & W., (1929, p. 49) mention that the definition may be made more precise by stating that a joint is a purely mechanical effect, a shearing or tearing, and it is distinct from the partings or tendencies to part that result from recrystallization. But Leith (1923, p. 29) is in favour of including 'rifts'—incipient joints—which are tight and inconspicuous along with joints. Where closely spaced tight joints give the rock a capacity to part along parallel surfaces, he calls the structure 'fracture cleavage'. At the same time all these structures have to be distinguished from 'faults', in which the essential feature is differential movement. In this respect, joints grade into faults. Also, as there is some displacement

along all joints, though it is sometimes not visible to the naked eye Daubree (Becker, 1894, p. 131) advocated that the term joint should be removed from the literature altogether. Nevin (1931, p. 138) points out that although faults may be microscopic in size, and the movement along fault surfaces therefore negligible, they should not be confused with joints. He says that the major movement in jointing is perpendicular to the fracture surface and this distinction is truly characteristic.

Joints usually occur in groups and differ very much in size and the interval between them may be hundreds of feet or only a few inches. In the latter case, Billings (1954) calls them 'fracture cleavages'.

Joints have also to be distinguished from other features like fracture and veins which occur in rocks in all directions.

Terminology of Joints:

Joints are usually arranged in 'sets' in the sense that all those joints that belong to one set have the same general strike and dip. A group of two or more intersecting sets of joints constitutes a 'joint system'. In most cases two or more sets of joints are observed rather than a single joint set. The blocks adjacent to joints or blocks bounded by joints are called 'joint blocks'. Their breadth depends upon the spacing of the joints. The opposing surfaces of two blocks at a joint are the 'walls' or 'surfaces' of the joint. Very often one set is more well developed than the other in which case, the better developed ones may be termed 'major set of joints'. The term 'master joints' is applied when major joints run for long distances.

In sedimentary rocks joints are described according to their relation to the attitude of the beds. Thus terms like strike joints, dip joints etc., have come into use. 'Strike joints' are those that strike parallel to the strike of the bedding of the sedimentary rock. 'Dip joints' are those that strike parallel to the direction in which the beds dip. 'Oblique' or 'diagonal' joints strike in any direction other than the two mentioned above.

When both the strike and dip sets exist or when two sets at right angles to each other are present, they are referred to as a 'conjugate system'. In general, a group of two or more intersecting sets of joints constitute a 'system'. (Leith 1923, p. 41-42) comments that there is lack of precision in this term. He says that systems

of joints may divide the rock mass symmetrically or asymmetrically. A system may be formed of a single episode of deformation or as a result of successive and unrelated episodes—often it is not possible to decide.

Joints are also described as either vertical, horizontal or otherwise according to their general magnitudes of dip.

There has developed an important set of terms in describing joints—according to their mode of origin. Thus, joints formed by compression are 'compression joints', those formed by tension are 'tension joints' and there are also other types like 'shear joints', 'torsion joints' etc. A number of characteristics which differentiate the compression joints from the tension joints have been described by different structural geologists. In contrast to compression joints, tension joints are apt to be irregular, uneven or rough on the parting surfaces and thus show that the rock was torn rather than cut apart. Compression joints have much smoother surfaces Becker (1905) tries to distinguish between the two and writes—"One species of fracture takes place by tension and is usually characterised by sharp curvatures and uneven surfaces; the mass is torn as under. The other method is by shearing motions due to pressure; the mass is cut to pieces by surfaces which are often and indeed characteristically flat and smooth." However, smooth joint surface does not necessarily prove shear origin.

The fact that the grain size of the rock also has a hand in determining the roughness of the joint surface, shows that these criteria are not always applicable. A fine-grained rock gives rise to a smoother joint surface than a coarse-grained rock.

Swanson (1927, p. 219) points out that the distinction between the two types is not simple. After an elaborate consideration of the stress and strain relations, he concludes that the terms compression and tension joints are often misinterpreted by the field geologist and proposes that joints be termed as 'slip' or 'rip' instead of as 'shear' (compression) or 'tension' thus not involving any reference to the stresses which produced them. He believes that this terminology would avoid encumbering the description of fairly simple facts with names which permit the possibility of unintended implications in the reader's mind.

'Torsion joints' are much similar to tension joints and they tend to gape in which case the term 'gaping joint' is also, sometimes

applied. Joints arranged in en echelon fashion are termed 'en echelon joints'. Where joints radiate from the center they are called 'radiating joints'.

Joints perpendicular to the axes of the folds, common in orogenic belts, are termed 'extension joints', and those parallel to the axial plane are termed 'release joints'. (Billings, 1954, p. 117).

'Sheeting' is another term which is usually used to represent a form of rupture similar to jointing. Sheeting surfaces are usually horizontal and sometimes somewhat curved and are essentially parallel to the topographic surface, except in regions where there has recently been rapid erosion. The interval between these joints is usually very small and it increases with depth. Sheeting is usually attributed to some type of tensional phenomenon due to the release of load during erosion.

In view of the complications arising in the nomenclature of of joints, some geologists have preferred to use numbers such as System I, System II, etc. This makes things much simpler. The description of joints in such terms as large, medium, small or very well developed, well developed, poorly developed, etc., are usually arbitrarily fixed by the local geology and according to the geologist who is working with them. In all such cases it would be very helpful if they specified the dimensions of their terms.

Some geologists have developed the idea of describing a particular joint set according to the direction of the strike of the set.

Classification of Joints:

The important factors that have to be taken into consideration while trying to classify joints are:

(1) their mode of origin,
(2) their attitude and relation to the attitude of the beds in which they occur,
(3) their age relations and
(4) others factors like their continuity, length, curvature, etc.

One of the earliest proposed classifications is the one based on origin. This classification is the outcome of a number of experiments conducted by people like Dauhree, Griggs, and others. They distinguished tension joints from compression joints. Apart from these they also noted those produced by shear and torsion. This classification is mainly based on theoretical approach and has also

been, to a large extent, applied in the field. However, much difficulty has been experienced in distinguishing one from the other and this is considered as a major defect. This led Swanson to propose terms which are non-comittal.

A second factor which has found much use by some geologists led to the classification of joints on a purely geometrical basis. Thus joints are termed as either strike joints, dip joints or diagonal joints. When the joints are essentially parallel to the bedding planes they are classified as 'bedding joints'.

This type of classification has the advantage that the terminology is not involved in the mode of origin which is usually difficult to decide. Hence this classification has found extensive use in normal field work.

The third mode of classification is to consider them on their age basis. The geologic age of joints may sometimes be ascribed by their relation to dikes, faults, folds or unconformities present in the area. The abrupt ending of one set of joints against the other is also helpful in determining the age relations between two sets of joints. However in the case of simultaneously developed joint sets such criteria may be absent.

In all these cases, the determination of the absolute age of the joint sets is difficult and at best they can be classified on a relative age basis. In this respect this type of classification is defective. Also, it does not give us any clue about the forces that gave rise to the joints.

On the basis of the other factors given under (4), joints could be classified as major or minor with respect to their length or continuity. When they are curved they could be classified as curved joints.

There are also other minor ways of classifying joints such as vertical joints, horizontal joints, low dipping joints, etc., but all these types can find use only as descriptive terms.

After a consideration of the advantages as well as the disadvantages of the various classifications that have been proposed it is pertinent to mention that not one of them is sufficient in itself. It might be pointed out that it is difficult to evolve a classification which takes into account all the possible factors without any complication.

In as much as the final intention of the study of joints is the understanding of the forces that acted on the area, it is best to study the various joints—according to their relation to the beds and their ages and from that try to conclude about the forces, rather than designate them on genetic basis right from the beginning. The genetic classification has to be applied only after a full evaluation of the various other factors described. Hence it is good to use all the classifications in conjunction with one another.

Conclusion:

In the light of the above discussion the following procedure is proposed for the classification of joints. First the joints have to be classified as either major or minor or curved or straight or well developed or poorly developed. Next they should be classified in terms of their relation to the attitude of the beds. With the above facts on hand the joints are next classified on their age relationships. Finally a phrase can be added, which would indicate the origin of the joints, if definitely known. To give an example one may describe a joint set as 'well developed—low dipping—strike joints of compressive origin', etc.

Though the nomenclature sounds too long and is not comprehensive it supplies all the information available on a set of joints under study.

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Leptynites of Pallavaram

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ABSTRACT

Two types of leptynites occur at Pallavaram. The field, microscopical and chemical characteristics reveal that cream-coloured leptynites are the migmatized products of khondalites due to the intrusion of charnockite and the bluish-grey garnetiferous leptynite has resulted owing to metamorphism of charnockite.

Field characteristics

Leptynite is a friable cream-coloured rock predominantly made up of quartz and felspar together with or without garnet. It occurs as distinct bands and lenses associated with khondalites and charnockites. East of Pallavaram railway station the leptynite band shows segregation of garnets which occur as lenses 1 foot long and 4 inches wide. It shows faint banding approaching that of a migmatite and often grades into khondalites on the one hand and charnockite on the other. At the contacts of charnockite there is gradation of colour from greyish-white to grey. It shows lineation both on fresh and weathered surfaces due to the elongation and directional orientation of quartz. In the bands of leptynite there is the frequent occurrence of country rocks.

Just east of Pallavaram railway station, in a low mound, south-west of Mosque Hill, patches and lenses of highly siliceous sillimanite gneiss occur as relics in the leptynite, and in the same mass radiating needles and streaks of sillimanite are seen plentifully distributed with haphazard orientation. Similar patches and lenses of varying sizes of sillimanite-gneiss occur most often in bands of leptynite, east and west of Pachaimalai, where, due to the presence of good exposures and contacts, they can be studied in detail. Along the contacts of sillimanite-gneiss garnet is abundantly developed in the leptynite which shows yellowish brown colour on

weathered surfaces due to the alteration of garnet to limonite. In the western portion of Pachaimalai bands of garnetiferous hedenbergite-magnetite-rock occurring in the leptynite are extensively ramified by quartz veins and show perfect banded structure due to the presence of alternating layers of quartz and magnetite with subordinate amounts of herdenbergite. The frequent occurrence of country rocks, and the presence of minerals of the country rocks, in the leptynite and its gradational passage to country rocks and charnockite indicate that the lepynite is a reconstituted phase of khondalite due to the intrusion of charnockite.

Petrography:

Petrographically leptynites can be subdivided into two types as follows:

1. Leptynite.
2. Garnetiferous leptynite.

Leptynite is a greyish white rock which, in thin Section (L 356), shows a typical gneissose texture due to the presence of elongated grains of quartz and drawnout stringers of perthite (Plate I, Fig. 1). Quartz occurs as broad plates and elongated grains with dusty inclusions and undulose extinction. Microcline perthite is present interstitial to quartz and is characterized by spindles of varying shape and size grouped at the centre. — 2V (variable) = 83° to 86° and $\times$ to (001) cleavage is + 10° on sections perpendicular to Z. It also occurs as lenticular patches and islands in the plates of quartz. Twinned grains of plagioclase are sparingly present and the twin laws determined on 12 grains gave 5 albite and 7 albite-ala laws. The anorthite content varies from 24% to 27% and a few grains show incipient sericitization. Biotite, pleochroic from deep-brown to yellow, occurs associated with iron ore and it wraps around magnetite at the contacts of microcline-perthite. Grains of calcite and epidote are rare as alteration products of plagioclase and rounded grains of zircon occur as accessories.

Garnetiferous leptynite is a medium-grained cream coloured rock characterized by quartz, felspar and garnet. In thin sections (L 359, 354) it has a xenomorphic granular texture and shows evidences of crushing. The principal components are quartz, microcline-perthite and antiperthite. Quartz contains acicular inclusions and shows crushing and granulation. Perthite occurs

FIG. 1

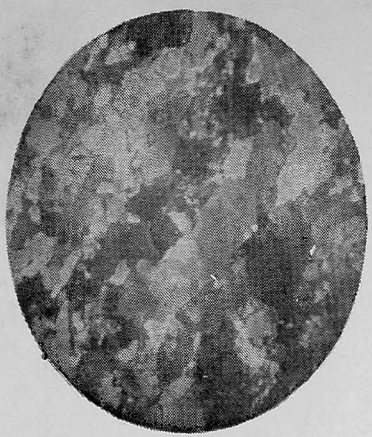

FIG. 2

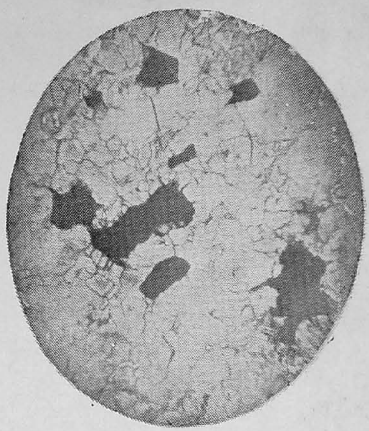

FIG. 3

FIG. 1. Elongated plates of quartz and crushed grains microcline-perthite in leptynite. × 30. × Nicols. FIG. 2. Tufts of silimanite along the margins of magnetite in leptynite. × 30. FIG. 3. Silimanite and microcline-perthite in plates of quartz in leptynite along the contacts of inclusions of garnetiferous silimanite gneiss. × 30. × Nicols.

as anhedral grains of the average size of 0·08 mm. × 0·20 mm. Soda felspar in the perthite is present as spindles of varying shapes in (010) sections and as blebs and irregular patches in (001) sections of microcline. The poles of the spindle axes appear near the emergence of c-axis on (001) plane of Nikitin's stereogram. In some cases the poles of the spindle axes are away from the emergence of c-axis, but usually lie, in the a-c zone. Usually one set of spindles are present and they are either parallel to murchisonite cleavage (15·0·2) or to albite twin plane (010). The spindles parallel to murchisonite cleavage make an angle of 71° with (001) cleavage on (010). Some grains show two sets of spindles and in such cases the spindles meet at an angle of 71° to 72° on (010) and at 90° on (100). When 'a' axis of the microcline in perthite is parallel to the axis of the microscope the spindles disappear and therefore it is concluded that α index of refraction of the host and guest in the perthite is the same. But β and γ of the microcline are lower than the corresponding indices of the spindles. The birefringences of fairly large irregular patches of the guest determined by computing the thickness with adjacent grains of quartz are $(N_g\text{-}N_p)=0\cdot007$; $(N_g\text{-}N_m)=0\cdot001$; $(N_m\text{-}N_p)=0\cdot005$. These values indicate that the spindles are really potash felspar rich in soda molecule and this is in accordance with the observation of Naidu (1955, P. 217). As the spindles are grouped towards the centre of microcline, and as they do not extend into the host from the margin, they are considered to be exsolution products. Antiperthite is sparingly present and the anorthite content of the plagioclase varies from 20% to 27% and the optic axial angle ranges from −74° to −82°. The twin laws determined on 25 grains are 12 albite law, 2 acline = Manebach ala, 3 pericline and 8 albite-ala laws. Garnet is pinkish in colour and occurs as rounded and irregular grains. The refractive index varies from 1·798-1·804. The percentage composition of garnet in terms of the standard molecule according to Naidu (1955, P. 253) is almandine 66·3%, grossularite 7·8%, pyrope 25·0%, and spessartite 0·9% and according to Howie (1955, P. 748) is almandine 77·6%, andradite 2·5%, grossularite 2·0%, pyrope 16·3% and spessartite 1·6%. Shreds of biotite occurs at the margins of garnet and magnetite is present as a minor accessory.

Nearer in, towards the contacts of Khondalites at Pachaimalai, the leptynite is much enriched with garnet. In slice (L 368) the most essential constituents are quartz, microcline-perthite and

plagioclase. Quartz is present as anhedral plates with dusty inclusions and shows effects of crushing. Microcline-perthite with one set of spindles crowded at the centre carries lumps and stringers of magnetite which occasionally show peripheral alteration to biotite. Plagioclass occurs as anhedral grains and is twinned on albite and albite-ala laws. The anorthite content varies from 24% to 30%. Myrmekite is sparingly present at the contacts of plagioclase and potash felspar. Garnet occurs as slightly pinkish porphyroblasts with sutured outline and inclusions of quartz, perthite and magnetite. Radiating tufts of sillimanite are present along the margins of magnetite (Plate IX, Fig. 2) and rounded grains of zircon are rare.

At the contacts of inclusions of sillimanite-gneiss in the leptynite in the low mound just east of Pallavaram railway station needles and prisms of silimanite occur in the cream coloured leptynite. In slice (L 372) it shows an imperfect gneissose texture due to occasional elongation of quartz grains. The most abundant minerals are quartz and microcline-perthite. Quartz occurs as anhedral plates with dusty and acicular inclusions and shows undulose extinction. Microcline perthite is present interstitial to quartz and sometimes carries blebs of quartz and prisms of sillimanite (Plate I, Fig. 3) which show alteration to an yellowish product. Garnet occurs as irregular pinkish plates with inclusions of quartz, perthite and sillimanite. Prisms and needles of sillimanite with cross-parting are abundant in the plates of quartz, in portions where microcline-perthite is very subordinate, and in places where microcline-perthite is abundant, only isolated prisms of sillimanite occur in the plates of perthite. Zircon and magnetite are present as minor accessories.

Inclusions of sillimanite gneiss in the leptynite is pale greyish white in colour and is highly quartzose with needles of colourless sillimanite and rounded grains of pink garnets. In thin Section (L 374) it is essentially made up of quartz, sillimanite and garnet. Sillimanite occurs as bunches in the plates of quartz which are elongated and characterized by dusty inclusions and undulose extinction. Porphyroblasts of pinkish garnet with inclusions of sillimanite and quartz are sparingly present and grains of magnetite and zircon are rare.

Besides the relics of sillimanite-gneiss there are also inclusions of garnetiferous-hedenbergite-magnetite-quartz rock and banded

quartz-magnetite rock in the leptynite band west of Pachaimalai. In slice (L, 377) banded quartz-magnetite rock is composed principally of quartz and euhedral and subhedral grains of magnetite with occasional grains of bluish green hedenbergite.

In order to trace the mineralogical variation from leptynite to the country rocks and charnockite the modal compositions of country rocks, leptynites and charnockite are shown in tables 1 and 2.

TABLE 1

Modes of paragneiss, leptynite and charnockite

Constituents	1	2	3
Quartz ..	63·7	38·9	43·3
Microcline perthite ..	19·4	49·0	45·7
Plagioclase ..	—	4·6	4·3
Biotite ..	0·3	0·5	—
Hypersthene ..	—	—	3·8
Garnet ..	6·6	5·3	—
Sillimanite ..	8·5	—	—
Ores ..	1·5	1·7	1·9
Total ..	100·0	100·0	100·0

1. Garnetiferous sillimanite gneiss.
2. Garnetiferous leptynite.
3. Charnockite.

TABLE 2

Modes of paragneiss, leptynite and charnockite

Constituents	1	2	3
Quartz ..	66·6	46·8	44·3
Microcline perthite ..	16·5	45·3	45·7
Plagioclase ..	—	3·6	4·3
Biotite ..	8·7	3·0	—
Hypersthene ..	—	—	3·8
Garnet ..	6·5	—	—
Ores ..	1·7	1 3	1 9
Total ..	100·0	100·0	100 0

1. Garnetiferous biotite gneiss.
2. Leptynite.
3. Charnockite.

Inspection of table 1 shows that there is decrease of quartz, sillimanite and magnetite as the leptynite is approached, and there is increase of microcline-perthite and plagioclase in the leptynite when compared to the country rocks. The only difference between the garnetiferous leptynite and charnockite lies in the presence of garnet in the former and hypersthene in the latter.

In table 2 the modal compositions of garnetiferous biotite-gneiss, leptynite and charnockite are presented. From the table it is evident that there are lower amounts of quartz, biotite and magnetite in the leptynite, when compared to the garnetiferous biotite-gneiss, and there is greater amount of perthite, and plagioclase in the leptynite than the country rock. There is no difference in the modal composition of leptynite and charnockite except that there is hypersthene in the latter.

The modal compositions shown in table 1 and 2 reveal the extreme quantitative mineralogical difference between the highly siliceous country rocks and the leptynites, but actually, there are country rocks which are equally felspathic as leptynites in the type area. This variation in the felspathic content of the country rocks makes some of the country rocks to approach very closely the mineralogical composition of leptynite, which closely resembles the mineralogical composition of charnockite with only subtle differences. Thus it is seen that the mineralogy of leptynites very closely approach some of the country rocks on the one hand and that of charnockite on the other.

A study of chemical variations between the country rocks, leptynites and charnockite requires a large number of analyses of the country rocks and leptynites. Analyses of fresh specimens of garnetiferous leptynite and leptynite and their norms and modes presented in table 3 together with the published analyses of related rock types from other parts of South India and their norms and modes reveal their close similarity. From the table it is evident that there is a slight discrepancy in the relative amounts of potash and soda felspars of the modes and norms of analysed specimens of the type area. This is due to the perthitic intergrowth of the entire alkali felspar. The normative plagioclase corresponds to the oligoclase variety and is in agreement with the optical data. The normative corudum and hypersthene are represented by garnet and biotite in the modes.

TABLE 3

Chemical analyses and norms of leptynites

Constituents	1	2	3	4
SiO_2 ..	72·95	76·63	74·36	74·28
Al_2O_3 ..	14·20	13·86	11·92	13·04
Fe_2O_3 ..	1·10	0·71	0·55	0·55
FeO ..	2·56	1·56	3·53	2·07
MnO ..	0·06	0·03	0·06	—
MgO ..	0·52	0·14	1·46	0·81
CaO ..	1·74	0·52	1·51	1·38
Na_2O ..	2 62	2 10	2·82	2·93
K_2O ..	3·73	4·27	3·57	4·62
TiO_2 ..	0·15	0·18	0·25	0·12
P_2O_5 ..	—	—	—	—
H_2O+ ..	0·11	0·06	0·11	0·57
H_2O ..	0·28	0·15	0·10	—
Total ..	100·02	100·21	100·24	100·37

Norms

Q ..	36·77	46·23	36·60	35·00
or ..	21·68	25·58	21·10	33 90
ab ..	22·01	17·82	23·60	18·30
an ..	8·34	2·50	7·50	5·80
C ..	2·86	4·79	0 71	0·30
hy ..	5·06	2·33	9·00	5·54
mt ..	1·62	0·93	0·90	—
il ..	0·15	0·46	0·60	0·70

1. Sp. L. 252. Garnetiferous leptynite. Pachaimalai, Pallavaram, Madras. Analyst. N. Leelananda Rao.

2. Sp. L. 261. Leptynite. Pachaimalai, Pallavaram, Madras. Analyst. N. Leelananda Rao.

3. Garnetiferous granulite. Dibbabodu Hill, Kondavidu Hill Range. Analyst. A. Narasinga Rao. (1950, p. 83).

4. Pink banded gneiss. One mile north of Kondappallee. Analyst. M. S. Rao (Narasinga Rao, 1950, p. 83).

Holland (1900, P. 143) considers the leptynites as metamorphosed members of charnockite and states "The rocks now referred to present the characters of those known to German petrographers as Saxon granulites, but rutile, kyanite, sillimanite etc., so frequently found in the Saxon granulites do not occur in

these rocks at Pallavaram." But the frequent occurrence of country rocks in the leptynite as revealed by the recent survey of the area by the author shows that the leptynites resemble closely the Saxon granulites.

The frequent occurrence of country rocks, the presence of needles of sillimanite with haphazard orientation, the occurrence of sills of basic and acid members of the charnockite series in the leptynite bands which show gradational passage to the country rocks on the one hand and to charnockite on the other reveal that they are the migmatized products of khondalites due to the intrusion of charnockite and this is in accordance with the observation of Krishnan (1950, p. 318).

There are also highly crushed bluish-grey garnetiferous leptynite derived from charnockite in the highly disturbed zone east of Pallavaram.

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Maximal Congruences on a Lattice

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ABSTRACT

Two concepts "lattice translate and effective interval" are introduced and made use of to get the intersection of maximal congruences on any semi-discrete lattice. It is shown that the intersection of maximal congruences on a modular lattice need not necessarily be the null congruence on it.

Introduction: The purpose of the present paper is to answer the question:—What is the intersection of maximal congruences on a lattice?

An answer to this question was given by M. H. Stone in the case of distributive lattices. He has shown that the intersection of maximal congruences on a distributive lattice is the null congruence on it. (Cf. M. H. Stone [3]*).

This paper solves the above question completely in the case of semi-discrete lattices. It is shown that the intersection of maximal congruences on any semi-discrete, weakly modular lattice L is the null congruence on L; while the intersection of maximal congruences on any semi-discrete lattice L is the weakly modular congruence on L.

The main results of the paper are listed below:—

(i) The intersection of maximal congruences on a semi-discrete, weakly modular lattice L is the null congruence on L.

(ii) Any congruence on L, a semi-discrete weakly modular lattice can be represented as an intersection of maximal congruences on L.

* The numbers in square brackets along with names refer to the reference at the end of the paper

(iii) The intersection of maximal congruences on a modular lattice L is not in general the null congruence on L.

(iv) In a semi-discrete lattice L the intersection of maximal congruences on L is the weakly modular congruence on L.

(v) Any congruence containing the weakly modular congruence on a semi-discrete lattice L can be represented as an intersection of maximal congruences on L.

This paper is divided mainly into three sections. In the first are listed the preliminaries. The second section deals with weakly modular lattices and maximal congruences; while the third section is a study of semi-discrete lattices and maximal congruences.

1. *Preliminaries*: The symbols $\leqslant$, $\not<$, $+$, $\cdot$ will denote inclusion, non-inclusion, sum (least upper bound) and product (greatest lower bound) in any lattice L; while the symbols $\subseteq$, $\cup$, $\subset$, ε will refer to set inclusion, union (set sum), intersection (set product) and membership respectively. Small letters a, b, ... will denote elements of the lattice while Greek letters θ, φ, ... will stand for congruences on the lattice.

A binary relation θ on L is said to be an *equivalence relation* if it satisfies:—

(i) $x \equiv x\ (\theta)$ (reflexive).

(ii) $x \equiv y\ (\theta) \Rightarrow y \equiv x\ (\theta)$ (symmetric).

(iii) $x \equiv y\ (\theta);\ y \equiv z\ (\theta) \Rightarrow x \equiv z\ (\theta)$ (transitive).

If it further satisfies the substitution property

(iv) $x \equiv y\ (\theta);\ x' \equiv y'\ (\theta) . \Rightarrow x+y \equiv x'+y'\ (\theta)$ then it is called an *additive congruence.*

An equivalence relation θ which has the substitution property

(v) $x \equiv x'\ (\theta);\ y \equiv y'\ (\theta) \Rightarrow xy \equiv x'y'\ (\theta)$; is called a *multiplicative congruence.*

If the binary relation θ satisfies conditions (i) to (v) then it is said to be a *lattice congruence* or merely a congruence on L. The congruences on a lattice form a complete lattice. (Cf. [1] p. 24).

The *sum* and *product* of an arbitrary family of congruences on a lattice are defined as follows:—

$a \equiv b\,(\cup \theta_i)$ if there exists a finite sequence $a=x_0, x_1, \ldots, x_n=b$ such that $x_{j-1} \equiv x_j\,(\theta_{ij})$ for some θ_{ij}, $j=1,2,\ldots n$; and $a \equiv b\,(\cap \theta_i)$ if $a \equiv b\,(\theta_i)$ for every i.

The null element and the unit element of the lattice of congruences on a lattice are referred to as the *null congruence* and the *unit congruence* of the lattice L respectively.

By the *interval* $I=(a, b)$ $(a \leqslant b)$ of a lattice L is meant the set $I=(x \mid x \varepsilon L;\ a \leqslant x \leqslant b)$.

An interval is said to be *proper* if it contains more than one element, and is called *prime* if it contains just two elements.

An interval (a, b) $(a \leqslant b)$ of L is said to be *annulled* by a congruence θ on L, if $a \equiv b\,(\theta)$. It is well known in a lattice that $a \equiv b\,(\theta) <=> ab \equiv a+b\,(\theta)$. Also $a \equiv b\,(\theta)$ in $L => x \equiv y\,(\theta)$ for all x, y such that $ab \leqslant x$, $y \leqslant a+b$. Thus an interval $I=(a, b)$ is annulled by θ if it belongs to a single class under the congruence θ on L.

Next if (a, b) is annulled by θ, all intervals of the type

$$(a+x_1,\ b+x_1)\ \&\ (ax_1,\ bx_1)$$
$$((a+x_1)x_2,\ (b+x_1)x_2)\ \&\ (ax_1+x_2,\ bx_1+x_2).$$
$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$
$$(((((a+x_1)x_2+x_3\ldots\ldots)x_n),\ (b+x_1)x_2)+x_3)\ \ldots.)x_n) \text{ and}$$
$$((((ax_1+x_2)+x_3)\ldots\ldots)x_n,\ ((((bx_1+x_2)x_3)+x_4)\ldots\ldots)x_n)$$

where *n is finite, are annulled by* θ. *All such intervals are called* lattice translates of (a, b). More generally an interval (c, d) $(c \leqslant d)$ is said to be a *lattice translate* of (a, b) if there exists a finite n such that $c=((((a+x_1)x_2)+x_3\ldots\ldots)x_n)$

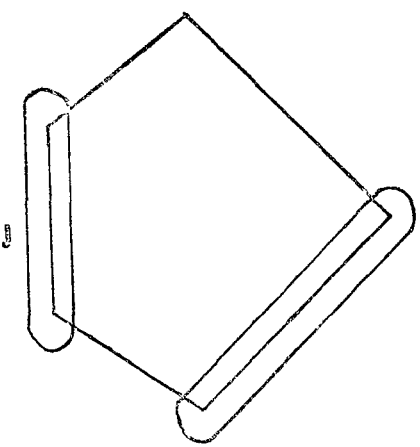
$$d=((((b+x_1)x_2)+x_3)\ldots\ldots)x_n)$$
$$\text{or } c=(((ax_1)+x_2)x_3)\ldots\ldots)x_n)$$
$$d=((((bx_1)+x_2)x_3)\ldots\ldots)x_n).$$

It is clear from the definition that any point is a lattice translate of all intervals and elements; and if (a, b) is annulled by θ all lattice translates of (a, b) are annulled by θ. The converse however is not always true. If a lattice translate of (a, b) is annulled by θ, (a, b) may or may not be annulled by θ. Next we ask the question; "If an interval I has J as its lattice translate, does there exist a proper interval I_1 of I such that J has I_1 as a lattice translate

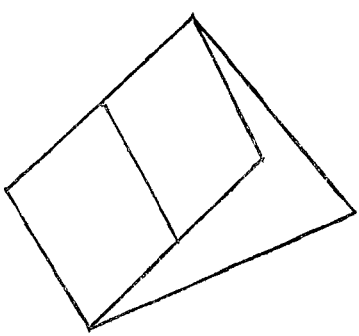
The answer to this question is in the negative, as is shown from figure 1; where J is a lattice translate of I but there exists no proper interval in I, which is a lattice translate of J.

Thus we are led to the definition of an effective interval. A proper interval is said to be an *effective interval* if whenever it is the lattice translate of an interval J, then there exists a proper interval J_1 in J such that J_1 is a lattice translate of I; whereas a proper interval which is not effective is said to be an *ineffective interval,* i.e., a proper interval is ineffective if there exists at least one interval J such that I is a lattice translate of J, but no proper interval J_1 of J is a lattice translate of I.

A lattice which has all its intervals effective is said to be *weakly modular.* Every modular lattice is weakly modular, but not conversely. Figure 2 gives an example of a weakly modular lattice which is not modular; hence the justification of the name "weakly modular" lattice.



TEXT-FIG. 1.



TEXT-FIG. 2.

A congruence θ_I on a lattice L is said to be a congruence *generated by an interval* I if θ_I is the smallest congruence which annuls I. Given I, the congruence θ_I can be defined thus: $x \equiv y\,(\theta_I)$ if and only if there exists a finite number of intervals $J_1, J_2, \ldots\ldots, J_n$ connecting x, y such that J_{i-1} J_i have a point in common, for $i=2, 3, \ldots\ldots n$; J_1, 3 x and J_n 3 y and each J_i is a lattice translate of I.

Now $x \equiv x(\theta_1)$ θ_1 is reflexive.

(ii) $x \equiv y(\theta_I) \Rightarrow y \equiv x(\theta_I)$ θ_I is symmetric.

(iii) $x \equiv y(\theta_I)$; $y \equiv z(\theta_I) \Rightarrow x \equiv z(\theta_I)\theta_I$ is transitive.

For $x \equiv y(\theta_I)$ implies there exists a finite number of intervals $J_1, J_2, \ldots\ldots, J_m$ connecting x, y such that J_{i-1} and J_i have a point in common for $i=2, 3, \ldots.$ m; and each J_i is a lattice translate of I. and $y \equiv z(\theta_I)$ implies there exists another finite number of intervals $J_{m+1}, J_{m+2}, \ldots, J_n$ connecting y, z such that J_{i-1} and J_i have a point in common for $i=m+2, m+3, \ldots, n$; and each J_i is a lattice translate of I. Now J_m and J_{m+1} have y in common Hence there exists a finite number of intervals $J_1, J_2, \ldots, J_n$ connecting x, z such that J_{i-1} and J_i have a point in common for $i=2, 3, \ldots, n$; and each J_i is a lattice translate of I. This implies $x \equiv z(\theta_I)$. Therefore θ_I is an equivalence relation.

Further $x \equiv y$ (θ_I) implies $x+z \equiv y+z$ (θ_I) for all $z \in L$. This follows by the fact that the intervals $J_1', J_2', \ldots, J_n'$, i.e., J_1+z, $J_2+z, \ldots, J_n+z$ connect $x+z$, $y+z$ and are lattice translates of I, and J'_{i-1} and J'_i have a point in common. Similarly $x \equiv y(\theta_I)$ implies $xz \equiv yz(\theta_I)$ for all z in L. Thus θ_I is a congruence relation on L annulling I.

Next if φ is any congruence annulling I then it annuls all lattice translates of I, and hence contains θ_I. Thus θ_I is the smallest congruence annulling I; i.e. θ_I is the congruence generated by I. Q.E.D.

Let (J_i) be the family of ineffective intervals of L. Let θ_J denote the congruence generated by J_i, for each J_i, and let $\psi = \Sigma\theta_{Ji}$. The congruence ψ on L is called the *weakly modular congruence* on L.

The weakly modular congruence on a weakly modular lattice is the null congruence on L.

A lattice L is said to be *discrete* if in L all bounded chains are finite, while if in L between each pair of comparable elements there exists a finite maximal chain, then we call the lattice semi-

discrete. It follows immediately that a discrete lattice is semi-discrete and a semi-discrete, modular lattice is discrete.

A lattice is said to be *weakly atomic* if whenever $a \leqslant b$ in L, then there exists elements u, v such that $a \leqslant u < v \leqslant b$; and v covers u; i.e., every interval in L contains a prime interval.

By the *pseudo-complement* of an element a in a lattice L with zero (0) is meant an element a′ such that (i) $aa' = 0$ and (ii) $ax = 0$ implies $x \leqslant a'$. The pseudo-complement of a congruence θ on L, considered as an element of the lattice of congruences on L is known as the *pseudo-complement of the congruence θ on L*. A lattice L in which every element has a pseudo-complement is called a *pseudo-complemented lattice.*

A congruence θ on L is said to be a *separable congruence* if between any pair of comparable elements $a \leqslant b$, we can find a finite sequence $a = x_0 \leqslant \ldots\ldots \leqslant x_n = b$ such that either $x_{i-1} \equiv x_i (\theta)$ or the elements in the interval (x_{i-1}, x_i) form single element congruence classes under θ. All congruences on a semi-discrete lattice are separable congruences.

A congruence θ on L is called a *maximal congruence on L* if it is covered by the unit congruence on L. One can easily see that if θ is a maximal congruence $\theta \lneq \varphi \lneq 1$ is impossible where φ is any congruence on L.

Lemma 1. If θ is a maximal congruence on L, then for any congruence φ on L, either $\varphi \subset \theta$ or $\theta \cup \varphi = 1$.

Proof: Now $\theta \leqslant \theta \cup \varphi \leqslant 1$. But θ is a maximal congruence of L, so there is no congruence between θ and 1. Hence $\theta \cup \varphi = \theta$ or $\theta \cup \varphi = 1$. In the former case $\varphi \subset \theta$ and in the latter case $\theta \cup \varphi = 1$.

Lemma 2. If θ is a maximal congruence, then $\theta \supset \varphi_1 \cap \varphi_2$ implies $\theta \supset \varphi_1$ or $\theta \supset \varphi_2$.

Proof: Let if possible $\theta \not\supset \varphi_1$ and $\theta \not\supset \varphi_2$. That is $\theta \cup \varphi_1 = 1$ and $\theta \cup \varphi_2 = 1$ (by lemma 1). Therefore $\theta \cup (\varphi_1 \cap \varphi_2) = (\theta \cup \varphi_1) \cap (\theta \cup \varphi_2 = 1$ (as the lattice of congruences on a lattice is distributive). Hence $\theta \not\supset \varphi_1 \cap \varphi_2$, a contradiction. Therefore either $\theta \supset \varphi_1$ or $\theta \supset \varphi_2$.

2. *Weakly modular lattices and maximal congruences.*

Consider a prime interval I of a weakly modular lattice L. Let (I_i) be the family of lattice translates of I in L. Then (i) I is a member of the family (I_i); for any interval is a lattice translate of itself; and (ii) any two intervals I_i and I_j of the family are lattice translates of each other. For I_i is a lattice translate of a prime interval I in a weakly modular lattice $L \Rightarrow$ a proper subinterval of I is a lattice translate of I_i (as I_i is effective, L being weakly modular). $\Rightarrow$ I is a lattice translate of I_i (being a prime interval a proper subinterval of I is I itself. $\Rightarrow$ I and I_i, are lattice translates of each other.

Similarly I and I_j are lattice translates of each other; and hence I_i and I_j are lattice translates of each other, and lattice translation is a transitive relation.

Lemma 3. If an interval J of a weakly modular lattice L contains a member of the family (I_i) then any interval K which has J as its lattice translate contains a member of the family (I_i).

Proof: Let J be a lattice translate of K, an interval in L. Let J contain I_i, a member of the family (I_i). As J contains I_i, I_i is a part of J and hence a lattice translate of J.

Now J is a lattice translate of K, and I_i is a lattice translate of J; therefore I_i is a lattice translate of K. Also I_i is effective, being an interval of a weakly modular lattice. Therefore a proper subinterval of K (say K_1) is a lattice translate of I_i. Now K_1 is a lattice translate of I_i and I_i is a lattice translate of I; hence K_1 is a lattice translate of I as well. Thus K_1 is a member of the family (I_i) and is contained in K. This proves the lemma.

Corollary 3. If an interval J of L, a weakly modular lattice, does not contain any member of the family (I_i), then any lattice translate of J does not contain any member of the family (I_i).

Lemma 4. If a chain C_1 joining x, y $(x \leqslant y)$ in a weakly modular lattice L contains a member of the family (I_i), then any other chain C_2 joining x, y contains a member of the family (I_i).

Proof: Let C_2 be a chain joining x, y let z be an element of C_2. Consider the intervals (x, z) and z, y). Let ξ and η be the congruences generated by (x, z) and (z, y) respectively. Then $x \equiv y (\xi \cup \eta)$; i.e., (x, y) is annulled by $\xi \cup \eta$, and so $\xi \cup \eta$ annuls every part of (x, y), in particular the interval I_j, which is a member of the family (I_i) contained in the chain C_1 joining x, y.

Implies $a \equiv b\ (\xi \cup \eta)$ where $I_j = (a, b)$. $\Rightarrow$ there exists a finite sequence $a = x_0, x_1, \ldots, x_n = b$ such that either $x_{i-1} \equiv x_i\ (\xi)$ or $x_{i-1} \equiv x_i\ (\eta)$ or $x_{i-1} \equiv x_i$ (both ξ and η). $\Rightarrow$ there exists a finite sequence $a = z_0 \leqslant z_1 \leqslant \ldots \leqslant z_n = b$ such that either $z_{i-1} \equiv z_i\ (\xi)$ or $z_{i-1} \equiv z_i\ (\eta)$ or $z_{i-1} \equiv z_i$ (both ξ and η); where $z_i = y_0 + y_1 + y_2 + \ldots + y_i$, and $y_i = (a + x_i)\, b$. This in turn implies either ξ or η or both annul a proper part of I_j say I_k). $\Rightarrow I_k$ is a lattice translate of either (x, z) or (z, y) or both.

But I_k is a part of I_j, hence is a lattice translate of I_j, also I_j is a lattice translate of I, therefore I_k is a lattice translate of I and hence a member of the family (I_i). Thus a member of the family (I_i), i.e., I_k is a lattice translate of either (x, z) or (z, y) or both. Now any interval in L and in particular I_k is effective, L being weakly modular. Hence a proper part of (x, z) or (z, y) or both is a lattice translate of I_k. That is either (x, z) or (z, y) or both contain a member of the family (I_i), which implies C_2 contains a member of the family (I_i).

Corollary 4. If a chain joining x, y $(x \leqslant y)$ does not contain any member of the family (I_i) then any other chain joining x, y cannot contain a member of the family (I_i).

Lemma 5. Define a binary relation on L, a weakly modular lattice, as follows: $x \equiv y\ (\theta)$ if and only if the convex sublattice $(xy, x+y)$ does not contain a member of the family (I_i); then θ is a congruence relation on L.

Proof: (i) $x \equiv x\ (\theta)$ $\theta$is reflective.

(ii) $x \equiv y\ (\theta) \Rightarrow y \equiv x\ (\theta)$ θ is symmetric.

(iii) $x \equiv y\ (\theta) \Rightarrow x+z \equiv y+z\ (\theta)$ for any z in L; θ is additive. For $x \equiv y\ (\theta)$ implies the convex sublattice does not contain a member of the family (I_i). Consider the convex sublattice $((x+z)(y+z), (x+z)+(y+z))$. Now this sublattice is a lattice translate of $(xy, x+y)$; for $(x+z)(y+z) = xy + (x+z)(y+z)$ and $(x+z)+(y+z) = (x+y) + (x+z)(y+z)$.

Therefore by virtue of corollary 3, the convex sublattice $((x+z)(y+z), (x+z)+(y+z))$ does not contain a member of the family (I_i). Thus $x+z \equiv y+z\ (\theta)$.

Similarly we have (iv) $x \equiv y\ (\theta) \Rightarrow xz \equiv yz\ (\theta)$ for any z in L. That is θ is multiplicative.

(v') Next $x \equiv y\ (\theta)$; $y \equiv z\ (\theta)$ with $x < y < z$ then $x \equiv z\ (\theta)$, for the convex sublattice (x, z) does not contain a member of the

family (I_i) as one chain joining x, z does not contain a member of the family (I_i) (by corollary 4).

(v) $x \equiv y(\theta)$; $y \equiv z(\theta) \Rightarrow x \equiv z(\theta)$ θ is transitive.

For $x \equiv y(\theta)$; $y \equiv z(\theta) \Rightarrow x \equiv x+y(\theta)$; $x+y \equiv x+y+z(\theta)$ (by (iii) above).

Also $x \equiv y(\theta)$; $y \equiv z(\theta) \Rightarrow xyz \equiv xy(\theta)$; $xy \equiv x(\theta)$ (by (iv) above).

Therefore $x \equiv y(\theta)$; $y \equiv z(\theta) \Rightarrow xyz \equiv x(\theta)$; $x \equiv x+y+z(\theta)$ $\Rightarrow xyz \equiv x+y+z(\theta)$ (by (v') above).
Implies the convex sublattice (xyz, x+y+z) does not contain a member of the family (I_i). $\Rightarrow$ the convex sublattice (xz, x+z) does not contain a member of the family (I_i), as (xz, x+z) is a part of (xyz, x+y+z). $\Rightarrow x \equiv z(\theta)$.

Thus θ is a congruence relation on L. Q.E.D.

Let θ_I be the congruence generated by the prime interval I on L, a weakly modular lattice. Let θ be the congruence relation on L defined as in Lemma 5. Then it is easily seen that (i) $\theta \cap \theta_I = 0$, and (ii) $\theta_I \cap \varphi = 0$ for any φ on L implies $\varphi \supset \theta$. Thus θ is the pseudo-complement of θ_I on L. Further if the lattice L is semi-discrete, then every congruence on L is separable and so is θ_I. Thus to every pair of comparable elements a, b$\in$L, a<b; either $x_{i-1} \equiv x_i(\theta_I)$ or (x_{i-1}, x_i) consists of single element congruence classes under θ_I. That is either $x_{i-1} \equiv x_i(\theta_I)$ or $x_{i-1} \equiv x_i(\theta)$. Which implies $\theta \cup \theta_I = 1$. Thus θ is the complement of the congruence θ_I on L.

Next let φ be any congruence on a semi-discrete, weakly modular lattice L. Let φ be greater than θ. Then φ annulls at least one member of the family (I_i) and hence every member of the family (I_i). Hence φ contains θ_I and so $\varphi = 1$, as it already contains θ. Thus the congruence θ as defined in lemma 5 is a maximal congruence on L, if L is semi-discrete besides being weakly modular. Thus we have,

Lemma 6. Given a prime interval I=(a, b) of a semi-discrete, weakly modular lattice L there exists a maximal congruence θ L such that $a \neq b(\theta)$.*

*For typographical convenience, we use $a \neq b(\theta)$ to mean a is not congruent to b (mod θ).

Lemma 7. The congruence θ generated by a prime interval $I=(a, b)$ of a distributive lattice L is a separable congruence on L.

This follows from the fact that $c<d$ and $c\equiv d\ (\theta)$ is impossible in a distributive lattice L if $b\leqslant c$ or $d\leqslant a$. For if $I_1=(a_1, b_1)$ $(a_1<b_1)$ is a lattice translate of $I=(a,b)$ in L, a distributive lattice, then $a_1=ay+z$ and $b_1=by+z$ for some y, z in L. (L being distributive, a lattice polynomial of finite length can be reduced to a lattice polynomial of length two.) Then $b\not< a_1$ and $b_1\not< a$.

For let if possible $b\leqslant a_1$. Now $ay+z=a_1$; $by+z\equiv b_1$ $z=a_1z=b_1z$. Next $b_1=by+z=by+a_1z$ (as $z=a_1z$).

$$=(by+a_1)\,(by+z)\quad\text{(as L is distributive)}.$$
$$=a_1b_1\quad\text{(as } b\leqslant a_1 \text{ by assumption)}.$$
$$=a_1\quad\text{(as } a_1<b_1).$$

This is a contradiction as $a_1\neq b_1$. Hence $b\not< a_1$. Similarly $b_1\not< a$.

Next if $c\equiv d\,(\theta)$ $(c<d)\Rightarrow$ there exists a finite sequence $c=x_o, x_1, \ldots . x_n=d$ such that (x_{i-1}, x_i) is a lattice translate of (a, b). Implies there exists a finite sequence $c=z_o\leqslant z_1\leqslant \ldots z_n=d$ such that (z_{i-1}, z_i) is a lattice translate of a, b); where $z_i=y_o+y_1+\ldots .+y_i$ and $y_i=(c+x_i)\,d$. Thus (c, z_1) and (z_{n-1}, d) are lattice translates of (a, b). So $b\not< c$ and $d\not< a$. Hence any chain joining a pair of comparable elements x, y in L can atmost contain one of the members of the family (I_i). Hence θ is a separable congruence on L.

Lemma 8. Given a prime interval $I=(a, b)$ of a distributive lattice L there exists a maximal congruence θ on L such that $a\neq b\,(\theta)$.

Proof follows on similar lines as for lemma 6.

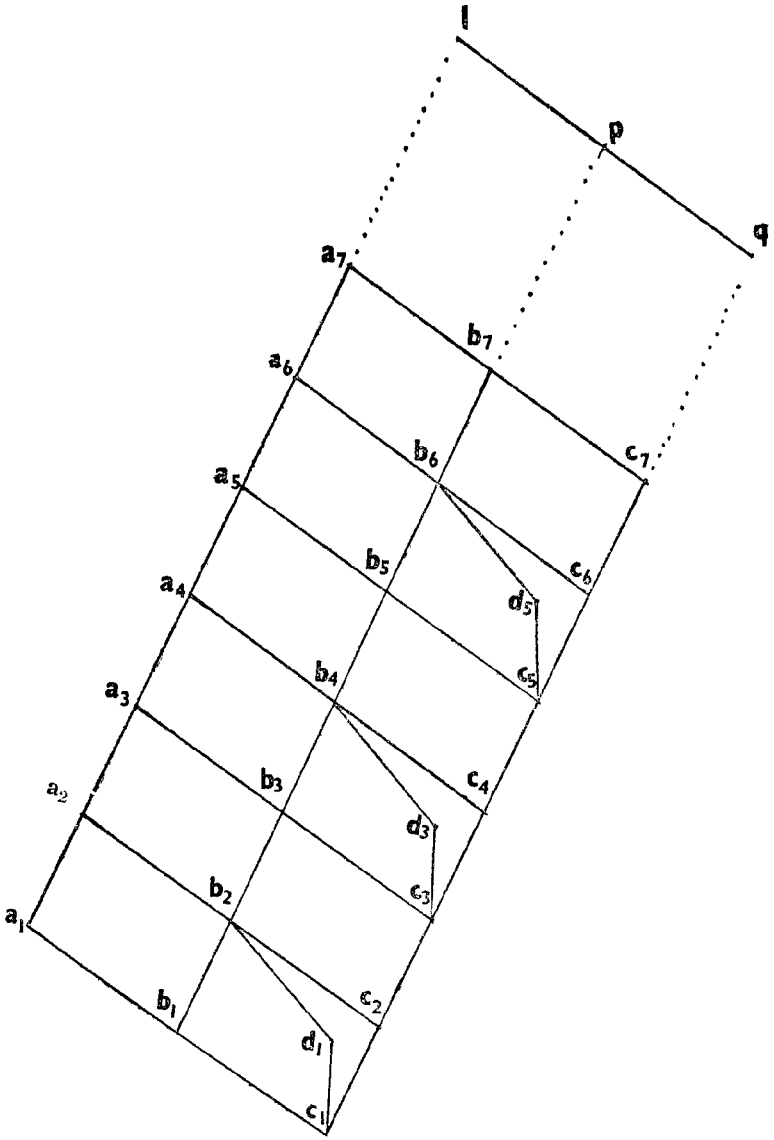
However the congruence on L generated by a prime interval I is not always separable; even when the lattice L is modular. For consider the lattice of Text figure 3. L contains ascending sequences (a_i), (b_i) and (c_i) such that $a_i/^1$, $b_i/^p$ and $c_i/^q$ and $a_i>b_i>c_i$ for each i. Consider the congruence on L generated by the prime interval (a_1, a_2). This congruence annulls (a_{2i-1}, a_{2i}) and does not annull (a_{2i}, a_{2i+1}) for $i=1, 2, \ldots .$ and so is not separable.

ERRATA

IQBALUNNISA, Maximal Congruences on a Lattice,
J. Madras Univ. 33B (2), 113-128, 1963

1. Page 114, line 14, read
"lower bound) in any lattice L; while the symbols $\subseteq, \cup, \cap, \varepsilon$" instead of, "lower bound) in any lattice L; while the symbols $\subseteq, \cup, \subset, \varepsilon$ | "

2. Page 121, line 18, read
"(i) $\theta \cap \theta_I = 0$ and (ii) $\theta_I \cap \varphi = 0$ for any φ on L *implies* $\varphi \subseteq \theta$" instead of, "(i) $\theta \cap \theta_I = 0$ and (ii) $\theta_I \cap \varphi = 0$ for any φ on L implies $\varphi \supset \omega$".


1
p
q
a7
b7
a6
b6
c7
a5
b5
c6
d5
a4
b4
c5
a3
b3
c4
d3
a2
b2
c3
a1
b1
c2
d1
c1

TEXT-FIG. 3.

Theorem 1. The intersection of maximal congruences on a semi-discrete, weakly modular lattice L is the null congruence on L.

Proof: It suffices to prove that there exists a maximal congruence θ_i on L for every distinct pair of elements a, b in L such that $a \neq b(\theta_i)$.

Now $a \neq b(\theta_i)$ (a, b in L $\Leftrightarrow$ $ab \neq a+b(\theta_i)$. That is to every pair of elements a, b in L with $a \neq b(\theta_i)$ there exists a pair (c, d) ($c<d$) in L with $c \neq d(\theta_i)$; namely, the pair ab, a+b. So it is enough if we prove that for every pair of elements c, d in L such that $c<d$, there exists a maximal congruence θ_i under which $c \neq d(\theta_i)$. Now if $c<d$ then there exists a pair of elements u, v such that $c \leqslant u<v \leqslant d$ and v covers u; as L is semi-discrete. By lemma 6, there exists a maximal congruence θ_i on L such that $u \neq v(\theta_i)$; which implies $c \neq d(\theta_i)$ for a maximal congruence θ_i. Thus for any two elements a, b in L there exists a maximal congruence θ_i on L such that $a \neq b(\theta_i)$. Hence the null congruence on L is the intersection of maximal congruences on L.

Corollary 1. The null congruence of a semi-discrete, modular lattice L can be expressed as an intersection of maximal congruences on L.

Corollary 2. The null congruence of a weakly atomic, distributive lattice L is the intersection of maximal congruences on L.

Proof on the same lines as in theorem 1.

Theorem 2. Any congruence φ on a semi-discrete, weakly modular lattice L can be represented as an intersection of maximal congruences on L.

Proof: Consider η the intersection of all maximal congruences $\theta_i \supset \varphi$ on L. Now $\eta \supset \varphi$. Next we prove $\eta \subset \varphi$

Let I be an interval of L not annulled by φ then there exists a prime interval $I_1 \subset I$ such that I_1 is not annulled by φ. By lemma 6 there exists a maximal congruence θ_i on L such that I_1 is not annulled by θ_i. Now $\theta_i \supset \varphi$. For let if possible $\theta_i \not\supset \varphi$ then by lemma 1 $\theta_i \cup \varphi = 1$. But θ_i does not annull I_1 and φ does not annull I_1 and I_1 is a prime interval, hence $\theta_i \cup \varphi$ cannot annull I_1. So $\theta_i \cup \varphi \neq 1$, a contradiction. Hence $\theta_i \supset \varphi$.

Thus any interval I is not annulled by φ implies a prime subinterval of I (namely I_1) is not annulled by a maximal con-

gruence on L containing φ. This in turn implies I is not annulled by a maximal congruence containing φ and hence I is not annulled by η. Thus $\eta \subset \varphi$, and so $\eta=\varphi$.

Remark. Any congruence on a weakly atomic, distributive lattice L can be represented as an intersection of maximal congruences on L.

Proof on the same lines as in theorem 2.

Lemma 9. The intersection of all maximal congruence on any lattice L contains the sum of all minimal nonseparable congruences on L.

Proof: Let φ be a nonseparable minimal congruence on L. Let if possible there be a maximal congruence θ such that $\theta \not\supset \varphi$. Then $\theta \cup \varphi = 1$, by lemma 1. Also $\theta \cap \varphi = 0$ as φ is a minimal congruence on L. Thus φ is complemented and θ is its complement. This implies φ is a separable, a contradiction. Thus every maximal congruence on L contains φ. This is true for all nonseparable minimal congruences φ. Hence the intersection of all maximal congruences on L contains the sum of all nonseparable minimal congruences on L.

Theorem 3. The intersection of all maximal congruences on a weakly modular lattice L is not in general the null congruence on L.

For there are minimal nonseparable congruences even on modular lattices. (See figure 3 and the congruence considered there).

3. *Semi-discrete lattices and maximal congruences.*

Lemma 10. Any maximal congruence θ on a lattice L annulls all ineffective intervals of L.

Proof: Let I be an ineffective interval of L. That is there exists an interval J of L such that I is a lattice translate of J but no proper subinterval of J is a lattice translate of I.

Let θ be a maximal congruence on L. Let if possible I be not annulled by θ. Then θ does not annull J as well. Consider the congruence $\varphi = \theta \cup \theta_I$ on L, where θ_I is the congruence generated by I.

Now (i) $\varphi \supset \theta$; since φ annulls I and θ does not annull I.

(ii) φ does not annull J, for J consists of single element congru-

ence classes under θ_1 and θ does not annull J. Therefore $\varphi \subset 1$. That is there exists a congruence between θ and 1; which contradicts our assumption that θ is a maximal congruence on L. Therefore θ should annull I; this is true for all ineffective intervals I of L. Hence θ annulls all ineffective intervals of L.

Corollary 1. The intersection of maximal congruences on a lattice L contains the weakly modular congruence on L.

Lemma 11. In a semi-discrete lattice L, if a prime I is a lattice translate of J then there exists a prime interval $J_1 \subset J$ such that I is a lattice translate of J_1. Further if I is an effective interval, J_1 is effective and I and J_1 are lattice translates of each other.

Proof: Let I be the interval (c, d) ((c<d) and d covers c). Let J be the interval (a, b). Let $a=a_0<a_1<a_2\ldots<a_n=b$ be a chain of length n connecting a and b such that a_i covers a_{i-1} for $i=1, 2, \ldots, n$.

Now I is a lattice translate of J. Therefore there exists a finite number of $x_1, x_2, \ldots, x_\gamma$ such that

$$c=((((a+x_1)x_2)+x_3)\ldots\ldots)x_\gamma) \text{ and}$$
$$d=((((b+x_1)x_2)+x_3)\ldots\ldots)x_\gamma).$$

Next for any i, $((((a_i+x_1)x_2)+x_3)\ldots\ldots)x_\gamma)$ is either c or d. if $((((a_i+x_1)x_2)+x_3)\ldots\ldots)x_\gamma)=c$ for $i=k$, then

$((((a_i+x_1)x_2)+x_3)\ldots\ldots)x_1)=c$ for all $i\leqslant k-1$. Similarly if $((((a_i+x_1)x_2)+x_3)\ldots\ldots)x_\gamma)=d$ for $i=p$, then for all $i\geqslant p+1$ $((((a_i+x_1)x_2)+x_3)\ldots\ldots)x_\gamma)=d$. Let m be the greatest value of i for which $((((a_i+x_1)x_2)+x_3)\ldots\ldots)x_1)=c$ and n be the smallest value of i for which $((((a_i+x_1)x_2)+x_3)\ldots\ldots)x_\gamma)=d$. Then it is easily seen that $n=m+1$. So (c, d) is a lattice translate of $(a_m, a_{m+1}) \subset a, b)$. Let $J_1=(a_m, a_{m+1})$. J_1 is a prime interval contained in J and has I as its lattice translate.

Next if I is effective then a proper interval J_2 of J_1 is a lattice translate of I. J_1 being prime $J_2=J_1$. Hence J, is a lattice translate of I. J_1 is effective, for let if possible J_1 be ineffective, then all lattice translates of J_1 are ineffective. But I an effective interval is a lattice translate of J_1, which gives rise to a contradiction. Hence J_1 is effective. Thus the lemma is proved.

Lemma 12. Given a prime interval (a, b) which is effective in a semi-discrete lattice L there exists a maximal congruence θ on L which does not annul (a, b).

Proof: Let I=(a, b) be the prime effective interval. Let (I_i) be the set of prime effective intervals such that I_i is a lattice translate of I for all i. Therefore I is a lattice translate of I_i for each i.

P. 1. Let $x<y$, x, y in L and C_1 be a chain joining x, y containing a member of the family (I_i) (I_j say). Then every chain joining x, y contains a member of the family (I_i).

Let C_2 be any chain joining x, y. Let z_2 be a point of C_2. Let ξ and η be the congruence generated by the intervals (x, z) and (z, y) respectively. Now $x \equiv y (\xi \cup \eta)$. Hence $\xi \cup \eta$ annulls I_j also. I_j being prime at least one of ξ or η annulls I_j. That is I_j is a lattice translate of (x, z) or (z, y) or both. I_j being an effective interval, there exists a prime effective interval J in (x, z) or (z, y) such that J is a lattice translate of I_j (by lemma 11). Hence J belongs to the family (I_i). Thus C_2 contains a member of the family (I_i). Thus we conclude:—If a chain joining x, y does not contain a member of the family (I_i) then the convex sublattice (x, y) does not contain any member of the family (I_i).

P. 2. If no member of the family (I_i) is contained in the interval (x, y) then no member of the family belongs to any lattice translate of (x, y).

Let if possible J a lattice translate of (x, y) contain a member I_j of the family (I_i). Then I_j is a lattice translate of J and hence a lattice translate of (x, y). By lemma 11 (x, y) contains a member of the family (I_i); a contradiction. Thus every lattice translate of (x, y) does not contain a member of the family (I_i).

Next define a binary relation θ on L as follows:—$a \equiv b (\theta)$ if and only if the convex sublattice $(ab, a+b)$ does not contain a member of the family (I_i).

Then θ defined above is a congruence relation on L. Proof on similar lines as in lemma 5, since P. 1. and P. 2. above give analogous properties for the family (I_i) as lemma 3 and lemma 4.

Further θ is a maximal congruence on L. For if φ is any congruence strictly containing θ, then φ annulls at least one of I or (I_i), and if it annulls one it annulls all and hence φ is the

unit congruence on L. Thus θ is a maximal congruence on L such that $a \neq b(\theta)$. This proves the lemma.

Theorem 4. The weakly modular congruence ψ on a semi-discrete lattice is the intersection of all maximal congruences on L.

Proof: Let η be the intersection of all maximal congruences on L. Now η contains ψ (by corollary of lemma 10).

Let J be any interval not annulled by ψ, then J contains a prime interval I, which is effective. Let if possible η annull J, then η annulls I, an effective prime interval of L. Now every maximal congruence on L contains η, hence every maximal congruence on L annulls I, a prime effective interval; which is a contradiction; as there exists a maximal congruence on L not annulling I (by lemma 12).

Thus if J is any interval not annulled by ψ then J is not annulled by η. Hence ψ contains η, which proves $\eta = \psi$.

Corollary 1. The null congruence on a semi-discrete, weakly modular lattice L is the intersection of all maximal congruences on L.

Theorem 5. Any congruence θ on a semi-discrete lattice L containing the weakly modular congruence on L can be expressed as an intersection of maximal congruences on L.

Proof on similar lines as in theorem 2.

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Neat Subgroups of Abelian Groups†

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SUMMARY

In this paper we study some of the properties of neat subgroups and related topics like 'N-high' subgroups. We investigate conditions which are necessary and sufficient for a subgroup to be a 'Neat Hull'. Many properties of subgroups seem to hold good also for their neat hulls. We consider the question of isomorphism of neat hulls and it is shown that under suitable conditions all neat hulls of a subgroup are isomorphic.

§1. A fundamental technique of group theory is to reduce the study of general groups to the consideration of groups of simpler structure. An important concept used in this connection is the notion of direct sums. Now if $G = G_1 + G_2$, then we can reduce the study of G to that of G_1 and G_2 which are called the *direct summands* of G. Thus direct summands are powerful enough to simplify structure-theoretical problems. But direct summands cannot always be obtained—as, for example, in the case of the large variety of indecomposable groups and in this connection the pure subgroups serve as an excellent substitute for direct summands. We say that S is pure in G if S is a direct summand of every intermediate subgroup I of G for which I/S is finitely generated. These are also characterised by the fact that for each integer n, whenever an element x of S is divisible by n in G, then it is also divisible by n in S. Pure subgroups have been extensively studied and constitute a powerful tool in the study of Abelian Groups. A weak form of this definition leads to the notion of neat subgroups as those in which the above criteria for pure subgroups is satisfied whenever n is a prime. Some simple properties of neat subgroups may be found in [1].

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Notation and Terminology: All groups considered in this paper are abelian. A subgroup N of a group G is neat in G, if $N \cap pG = pN$ for every prime p. If G is any group, then G^1 denotes the subgroup of all elements of infinite height in G, (i.e.) $G^1 = \cap nG$. we call G^1 as the *Radical* of G. Let N be a subgroup of G. Then a subgroup H maximally disjoint with N is called a N-high subgroup of G. If $N = G^1$, H is called a high subgroup of G. A subgroup S is *dense* in G if every nonzero subgroup of G intersects S. It is easy to see that if N is neat and also is dense in G, then $S = G$. A minimal divisible group containing G is called a divisible hull of G. For general notation used here we refer to [1]. The subgroup $\varphi(G) = \cap pG$, where p runs over all primes is called the Frattini subgroup or the φ-subgroup of G.

§2. The following lemma gives simple characterisations of neat subgroups which can be easily verified.

Lemma 1: (i) A subgroup S is neat in G iff every coset modulo S of prime order contains an element of the same order as the coset.

(ii) S is neat in G iff S is a direct summand of every 'minimal extension' I in G, (i.e.) of every intermediary subgroup I of G such that I/S is prime cyclic.

Theorem 1: If N is a subgroup of G, then a subgroup B is N-high in G iff B is neat, $B \cap N = 0$ and $\{B, N\}$ is dense in G.

Proof. If B is N-high, then it is known (p. 92, [1]) that B is neat and $B \cap N = 0$. We have only to show that $\{B, N\}$ is dense in G. If S is any subgroup of G, then S should intersect $\{B, N\}$, otherwise, S will, in particular, be disjoint with both B and N, so that the group $\{S, B\}$ is then disjoint with N and contains B, in contradiction to the maximality of B. Hence $\{B, N\}$ should be dense in G.

Conversely, if B satisfies these conditions, then B should be N-high. In fact, if $B' \supset B$ and $B' \cap N = 0$, then $\{B, N\} = B + N$ is dense in $\{B', N\} = B' + N$, which means that B is dense in B' and since B is neat we have that $B = B'$.

Remark: If G is torsion, the above condition can be replaced by (i)' B is neat and $B[p] + N[p] = G[p]$ and this is proved in [2].

The following theorem characterises neat subgroups in terms of 'N-high' subgroups, the proof of which is easily verified.

Theorem 2: Let N be a subgroup of G. Then the following are equivalent:

(i) N is neat in G.

(ii) For some N-high subgroup H, N is H-high in G.

(iii) For every N-high subgroup H, N is H-high in G.

We now prove a theorem regarding the neat subgroups of a group G.

Theorem 3: Every neat subgroup of G contained in the Frattini subgroup of G is a direct summand.

Proof. Let N be neat in G, such that $N \subseteq \varphi(G)$. Let $a \in N$ and p be any prime. Since a is in the φ-subgroup of G, it is divisible by p in G. But N is neat and so a is divisible by p in N. Since a is arbitrary, it follows that every element of N is divisible by p in N; (i.e.) $pN = N$. Thus N is divisible and hence is a direct summand of G.

Corollary: (i) A neat subgroup N of G is divisible iff $N \subseteq \varphi(G)$.

(ii) The φ-subgroup of a torsion group G is neat in G iff G is a direct sum of a divisible group and an elementary group.

Remark: Since every neat subgroup contained in $\varphi(G)$ is divisible, it is worth noting that a neat subgroup N of G will be contained in $\varphi(G)$ iff it is contained in G^1. In particular, if G is without elements of infinite height, then 0 is the only neat subgroup of G contained in $\varphi(G)$.

§3. *Neat Hulls:*

In this section we will study the notion of neat hulls which are analogous to the divisible hulls.

Definition: A neat subgroup N minimally containing a subgroup S of a group G is called a *Neat Hull* of S.

An important known result is that every subgroup possesses neat hulls (p. 92, [1]). If S is a subgroup of G then a neat hull of S is the intersection of G with a divisible hull of S. If G is torsion free, then there is a unique neat hull of S.

The following theorem gives a few characterisations of neat hulls of a subgroup.

Theorem 4: Let S be a subgroup of a group G and $N \supset S$. Then the following are equivalent:

(i) N is a neat hull of S.

(ii) N is neat and S meets every subgroup of N, (i,e.) S is dense in N.

(iii) N is neat and there exists a subgroup H which is high with respect to both N and S.

(iv) N is a maximal subgroup having S as a dense subgroup.

Proof: That (i) $<=>$ (ii), follows from Kulikov's theorem (see [1]) on divisible hulls and the fact that N is the intersection of G with a divisible hull of S.

Now (iii) implies (ii). For, if H is S-high as well as N-high, then, by theorem 1, $H + S$ is dense in $H + N$ which means that S is dense in N.

Similarly, we can check up that (ii) $=>$ (iii).

Now assume (iv). We will prove (ii) Since S is dense in N, it is enough if we prove that N is neat. But this follows immediately from the maximality of N and the fact that any neat hull of N would also contain S as a dense subgroup. Hence (iv) implies (ii).

A similar argument will show that (ii) $=>$ (iv) and this completes the proof.

Remark: (a). The condition (ii) implies that if N is a neat hull of S, then S contains every prime cyclic subgroup of N.

(b) If G is a torsion group, then (ii) and (iv) can be relaxed to the following two conditions:

(ii)′ N is neat and $N[p] = S[p]$ for each prime p.

(iv)′ N is maximal subgroup having, for each prime p, $S[p]$ as its p-socle.

Corollary: If N is a neat hull of S, then every S-high subgroup is N-high and further, if B is A-high, then every neat hull of A is B-high.

In the following theorem we consider neat hulls in a direct sum.

Theorem 5: Let $G = \sum G_\alpha$. Let, for each α, N_α be a neat hull of a subgroup S_α in G_α. Then $N = \sum N_\alpha$ is a neat hull of $S = \sum S_\alpha$ in G.

It turns out that a subgroup depends on its neat hull for many of its properties. To mention a few, a subset is maximal independent in S iff it is maximal independent in N. Thus the rank of S is completely determined by knowing the rank of its neat hull. In fact, $r_0(S) = r_0(N)$ and $r_p(S) = r_p(N)$ for each prime p. A subgroup A is disjoint with S iff it is disjoint with N. So all S-high subgroups are determined once we know all N-high subgroups. If G is torsion, then S is decomposable iff N is decomposable and S is cyclic iff N is of rank 1.

In the following theorem S stands for a subgroup of G and N is its neat hull.

Theorem 6: A subgroup N^* is neat in S iff it is of the form $N^* = S \cap N'$, for some neat subgroup N' of N.

Proof: Let N' be neat in N. Consider $N^* = S \cap N'$. Let $px = a \in N^*$, where $x \in S$. Since $a \in N'$, neatness of N' will imply that $px = py$, $y \in N'$, so that $p(x - y) = 0$. Then $(x-y) \in N[p] = S[p]$ which will mean that $y \in S$ and hence to N^*. Thus N^* is neat in S.

Conversely, if N^* is neat in S, then we can easily prove that $N^* = S \cap N'$, where N' is a neat hull of N^* in N. This concludes the proof and we have shown that all neat subgroups of S are determined by all the neat subgroups of its neat hull N.

Corollary: (i) Let S' be a subgroup of S. Then any S'-high subgroup H' in S can be represented as $H' = H \cap S$ where H is a S'-high subgroup in N and conversely.

(ii) Two neat subgroups of S are mutually high iff their 'defining' neat subgroups in N are mutually high in N.

§4. *Isomorphism of neat hulls*:

If D and D' are any two divisible hulls of S, then we know that there exists an isomorphism between D and D'. Unfortunately, it turns out, as the following example shows, that two neat hulls of a subgroup S are not, in general, isomorphic.

Example: Let $G = A + B$, where $A = c(p^\infty) = \{a_1, a_2, \ldots, a_n, \ldots\}$ with $pa_1 = 0$ and $a_{n-1} = pa_n$ and $B = c(p) = \{b\}$. Then we can check up that the subgroup A as well as the subgroup $A' = \{(a_2 + b)\}$ are neat hulls of the subgroup $\{a_1\}$. But clearly A is not isomorphic to A'.

Remark: In [2] it has been asked whether two pure subgroups of a torsion group having the same socle are isomorphic. The above example answers this question in the negative when we take one of the subgroups concerned to be neat.

In the following we are going to consider conditions under which any two neat hulls of a subgroup are isomorphic. Incidentally we will be considering the question of isomorphism of high subgroups of a group. Before that we prove a priliminary lemma.

Lemma 2: If G is characteristic subgroup of one divisible hull D of G, then it is characteristic in every one of its divisible hulls.

The proof follows on noting that if D' is any other divisible hull of G, then there exists an isomorphism θ of D to D' such that $\theta G = G$ elementwise and that for any automorphism δ of D', $\theta^{-1}\delta\theta$ is an automorphism of D.

Theorem 7: All neat hulls of any subgroup S of a group G are isomorphic if either G is divisible, or, more generally, G is a characteristic subgroup of its divisble hull.

Proof: If G is divisible, then all neat hulls of S are its divisible hulls and so are isomorphic.

If G is not divisible, let it be a characteristic subgroup of its divisible hull E. Let N and N' be two neat hulls of S in G and D and D' be the corresponding divisible hulls of S in E. Then there exists an isomorphism θ of D to D' such that $\theta(S) = S$, elementwise. Let D* be a divisible hull in E of a S-high subgroup. Then clearly $E = (D + D^*) = (D' + D^*)$. Now the mapping α of E in to itself defined in such a way that $\alpha(D) = \theta(D)$ and $\alpha(D^*) = D^*$ elementwise, can be easily seen to be an automorphism of E and since G is characteristic in E, α is an automorphism of G also. Since α maps D onto D', it maps $D \cap G$ onto $D' \cap G$. In other words α maps N onto N' and since it is an automorphism of G, it follows that N is isomorphic to N'.

Remark: It is clear that under these conditions on G, any two neat hulls of S are 'conjugate', (i.e.) there always exists an automorphism of G which carries N to N'.

Corollary: Let S be a subgroup of a group G, where G is divisible or is a characteristic subgroup of its divisible hull. Then all S-high subgroups are isomorphic.

Conclusion: The author wishes to express his grateful thanks to Prof. M. Venkataraman to whom he is indebted for his comments, criticisms and help in preparing this paper. He also thanks Prof. E. A. Walker for having given him the benefit of paper [2] long before publication.

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The Physiology of Toxin Action and Defence Reactions in Infectious Diseases of Plants*

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I consider it a great privilege and honour to have been invited to deliver these two lectures of the Dr. Todla Ekambaram Endowment under the auspices of this University. I take this opportunity to express my gratitude to my teacher, Professor Dr. T. S. Sadasivan, the President of this evening, who initiated me into the study of plant pathology over a decade ago and who nurtured me in those formative stages, and to Professor Dr. Ernst Gäumann who has influenced my thought considerably in recent years. Some of the ideas I am putting forward here are the result of listening to his lectures and the discussions I had the privilege to have with him.

I. PHYSIOLOGY OF TOXIN ACTION

The study of plant disease is centuries old, but Plant Pathology as a science has not made much progress, and is today what human medicine was some decades ago. Professor Brierley (1946) aptly summarises the position thus: "Up to the present the study of plant disease has remained mainly an increasing aggregate of data, techniques and expertize relating to circumscribed area of natural processes and phenomena. It has not attained the status of a science, a 'universe of discourse', for such an aggregate only becomes a science in so far as it develops a consistent body of theory which correlates the facts into logical and explanatory system". The first serious attempt to develop the necessary theory has been made by Gäumann (1946) in his book *Pflanzliche Infektionslehre*. According to Brierley, it is essentially a treatise on the theoretical foundations of plant pathology and it takes a long and decisive step towards the establishment of plan pathology as a science.

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Thus the plant pathologists, infants as they are, try to draw their ideas and inspiration from the field of human diseases. In my opinion, it is good to introduce ideas from one field of human endeavour to another, but only, we should not draw absolute conclusions without a critical evaluation.

Concept of the Term 'Toxin'

Let us for example take the term 'toxin'. Actually we have borrowed it from the conceptual sphere of human medicine. The idea that infectious diseases might be caused by microbial poisons was entertained even before the germ theory of disease was established. Both endo- and exotoxins were foreseen by Vallisnieri in 1713 (van Heyningen, 1955). Klebs, in 1872, had visualised the possibility of toxins playing a role in infectious diseases while Loeffler who discovered the diptheria bacillus in 1884 got evidence to show that the bacterium produced a poison in the host tissues (van Heyningen, 1955). However, the credit for the discovery of toxin is generally given to Roux & Yersin (1888), of whom the former was a student of Louis Pasteur. Roux (1889) also stated that by administering gradually increasing doses of the toxin to animals, in such a way as gradually to accustom them to their presence, it was possible to render them refractory, not only to toxic doses of the poison which originally would have caused death, but even to the microbe itself. Subsequently, von Behring & Kitasato (1890) discovered tetanus toxin and a few years later van Ermengem (1896) discovered the highly potent bacterial poison, the toxin of botulism.

Thus, a toxin, as we understand now in human pathology, is a protein, has antigenic properties and also a definite antitoxin. In fact, these characters differentiate these substances of microbial origin from real poisons. It is also clear that the term toxin implies in itself toxicity *in vivo*. Nevertheless, we should remember that all these attributes were little known when Roux first visualised the presence of a toxin in the disease caused by *Corynebacterium diphtheriae* (Flügge) Lehet N. In the diseased animals he observed symptoms in regions far away from the seat of infection. This, he thought, might not be due directly to the microbe itself but rather to its metabolic products that have been translocated. It was only subsequently, with the discovery of toxins in other diseases, that the term 'toxin' took concrete shape as we understand today.

Toxins in Plant Diseases

If one poses the question "Is there a toxin known to plant pathology?", the answer has to be made with a certain amount of reservation. However, if we were to approach the problem in the same spirit as Roux, then we do have toxins in infectious diseases of plants. In plants, symptoms are known to be produced in regions far away from the focus of infection and the most outstanding examples are the vascular wilts. Here the seat of infection is the root and the pathogen invades at the most the stem, while the pathogenic manifestations are seen in the leaves; hence the term 'wilt toxins' and the concept of toxins in plant diseases. Thus, in principle, toxins are known in infectious diseases of plants. In the course of future researches, we might come across toxins true to the letter and spirit of the word. Perhaps, the discovery of a toxin in the wildfire disease of tobacco is the first step in this direction. This disease, however, is caused by a bacterium and naturally bears a certain similarity to diseases in man and animals, the majority of which are of bacterial origin. But the majority of diseases of plants are of fungal origin and much remains to be done in this domain.

I have ventured to give this long introduction in an effort to remove certain ambiguities in the current literature and also the doubts in the minds of some as to the propriety of the term 'toxin' now current in plant pathology.

The parasites causing diseases of plants damage their hosts mostly through their 'enzymes' that cause dissolution of the host material and 'toxins' which cause functional disturbances in the host. In principle this can be shown, but in practice it is difficult to demarcate the two complex phenomena. In this lecture, I propose dealing only with the toxin component.

Chemical Nature of Toxins

In Table 1 are named a few toxins, known to be produced by plant parasites, which chemically belong to different groups of substances. These substances bear no relationship either to the systematic position of the causal agents that produce them or the hosts that they parasitise. These substances have been discovered by those interested in studying and demonstrating the role of toxins in pathogenesis, or those interested in finding out new antibiotic substances, with biological control or chemotherapeutic control of

TABLE 1

Details about some of the known phytotoxins

Causal agent	Host of choice	Toxin	Chemical nature	Molecular weight
Fusarium lycopersici Sacc.	Tomato	Lycomarasmine	Dipeptide	277
Fusarium lycopersici Sacc.	Tomato	Fusaric acid	Pyridine-carboxylic acid	179
Fusarium oxysporum Schlecht	Potato	Enniatin 'A'	Polypeptide	455
Penicillium patulum Bain	Potato	Patulin	Lactone	154
Alternaria solani (Ell. *et* Mart.) Sor.	Potato	Alternaric acid	Dibasic acid	410
Endothia parasitica (Murr.) And. & And.	Chestnut	Diaporthin	Bianthraquinone	250
Pseudomonas tabaci (Wolf & Foster) Stev.	Tobacco	Wildfire Toxin	A new type of amino acid	206

diseases in view. Some of these antibiotics have also proved to be toxins capable of inducing wilt in plants *in vitro*. All these substances termed toxins are chemically simple molecules with low molecular weights, in contrast to the complex molecules of the classical toxins known in human medicine. For example, the molecular weight of diphtheria toxin is 72,000 and that of the toxin of botulism 900,000. Because of their small size and simple structure the phytotoxins are extremely mobile in biological systems, and they reach the subcellular structures where they interfere with their functions. These substances have been described as chemically active toxins by Gäumann (1951). The only substance that we know of till now, which acts by virtue of the large size of its molecule and hence called a physically active toxin is glucosan. This is known to be produced by *Pseudomanas solanacearum* Erw. Smith, the causal organism of slime disease of tobacco and groundnut.

Specificity of Production

It is of interest to know the specificity regarding the production of these substances by the parasites. The answer to the question, 'Is a specific toxin produced only by a specific parasite?' is in the negative. Enniatin A, an endotoxin, is produced by *Fusarium sambucinum* Fuck., *Fusarium avenaceum* (Fr.) Sacc. and *Fusarium scirpi* Lamb. and Fautr. in addition to *Fusarium oxysporum* Schlecht. Similarly the exotoxin fusaric acid is produced by *Fusarium lycopersici* Sacc., the tomato wilt organism, *Fusarium vasinfectum* Atk., the cotton wilt fungus, *Fusarium heterosporum* Nees, a plurivorus parasite, *Gibberella fujikuroi* (Saw.) Woll., causal organism of 'Bakanae' disease of rice plants, and the saprophytic *Nectria cinnabarina* (Tode) Fr. The only exception is lycomarasmine hitherto known to be produced only by *Fusarium lycopersici* Sacc.. Perhaps this is true of wildfire toxin also. However, in the case of human pathogens the situation is different. There is a high degree of specificity between a pathogen and the toxin it produces: tetanus toxin is specific to *Clostridium tetani* (Flügge) Holland and the diphtheria toxins to *Corynebacterium diphtheriae* (Flügge.) L. *et N.*. In this perspective the parasites of plant disease appear to be more primitive or perhaps we can say they are not so well specialised.

If we were to ask the question 'Does a specific parasite produce only a specific toxin?' again the answer is in the negative.

For example, *Fusarium lycopersici* Sacc. is known to produce, in addition to lycomarasmine, fusaric acid and vasinfuscarin *in vitro*. This problem has not yet been thoroughly worked out, but we have reasons to believe that this may be true of other parasites also. In the case of pathogenic bacteria of warm blooded animals there are examples of more than one toxin being produced by a parasite inside the host: *Clostridium welchii* Holland, which causes gas gangrene in man, produces no less than four toxins and some of the haemolytic streptococci also produce more than one toxin.

Selective Action

Do these toxins act uniformly on all the tissues of the host? Evidence so far obtained shows that these toxins have certain selective predilection for their host tissues. The toxins lycomarasmine and alternaric acid act on the intercostal fields without affecting the vascular bundles of the leaves (Plate 1, Fig. 5) when administered *in vitro*, whereas patulin, diaporthin and fusaric acid cause necrosis of the vascular bundles (Plate 1, Fig. 1). Wildfire toxin produces chlorotic halos in the leaves (Plate 1, Fig. 6).

This selective predilection of toxins for host tissues might not be so refined or so highly specific as our experiences have shown with toxins affecting man and other animals. This is due to the fact that in plants the tissue organisation has not reached that level of differentiation as in man and animals, in addition to the fact that they lack a blood vascular system. It must be also borne in mind that in plants, unlike in animals, ageing is a dual process—age of the individual organs and age of the plant as a whole. It is known that tissues of organs of different ages show varying degrees of sensitivity to the same concentration of the toxin.

Range of Action

The parasites producing the toxins might be discussed under two groups, namely, those with long range action and those with short range action.

In the parasites of the first group, the site of infection and the regions showing the disease symptoms are far removed from each other. In medicine, tetanus is the standard example. The pathogenic bacterium develops anaerobically in a peripheral wound and its toxin penetrates into the host tissue, diffusing along the nerve tracts to the brain and there producing the characteristic paralysis of the motor system. In the plant world, this group is represented by a series of economically important wilt diseases caused by

many species of *Fusarium* and *Verticillium*, *Endothia parasitica* (Murr.) And. & And., and a few others. In all these cases the parasite attacks the roots, or at the most the stems, and transmits its toxins through the vascular system into the shoots.

In the parasites of the second group the toxins operate directly on the tissues surrounding the focus of infection. Not only the parasite, but also the toxin it produces and the damage done are localised; the site of disease coincides very largely with the site of infection. In medicine this type of infection is very rare. *Clostridium histolyticum* (Weinberg & Seguin) Bergey *et al.*, a wound parasite, causes necrosis at its point of attack, and dissolves the tissues down to the skeleton. In plants, on the other hand, numerous diseases are of this type—for example most of the leaf spot diseases.

I shall be taking up for discussion the mode of action of wildfire toxin produced by *Pseudomonas tabaci* (Wolf & Foster) Stev. as representing a toxin from a parasite with short range action, after presenting the mode of action of fusaric acid, a wilt toxin, as representing a toxin from a parasite with long range action.

Toxin from a Parasite with Long Range Action

Fusaric acid (5-n butylpyridine-2-carboxylic acid) (Text fig. 3, V) was first isolated from the fungus *Gibberella fujikuroi* (Saw.) Wr. by the Japanese workers Yabuta, Kambe and Hayashi in 1934 and was christened as fusarinic acid. Many years later it was reinvestigated after isolation from the tomato wilt fungus *Fusarium lycopersici* Sacc., and was recognised to be an important wilt toxin by Gäumann (1957, 1958). I shall confine myself to describing the mode of action of this toxin which is now known to play a role in pathogenic wilt of cotton, tomato and banana plants (Kalyanasundaram & Venkata Ram, 1956; Kern & Klüpfel, 1956 and 1959).

Mode of Action of Fusaric acid on Plant Protoplasts

In the study of mode of action of a drug or a toxin, it is customary to choose unicellular organisms as test objects. Since the majority of these organisms grow and reproduce quickly, any action of the toxin on their multiplication or growth can be easily demonstrated and quantitatively measured. The toxin or antibio-

tic is also absorbed more uniformly by the microorganisms growing in the nutrient medium than by higher plants. Sometimes the study is also conducted with specific functional systems of the host plants or related plant species.

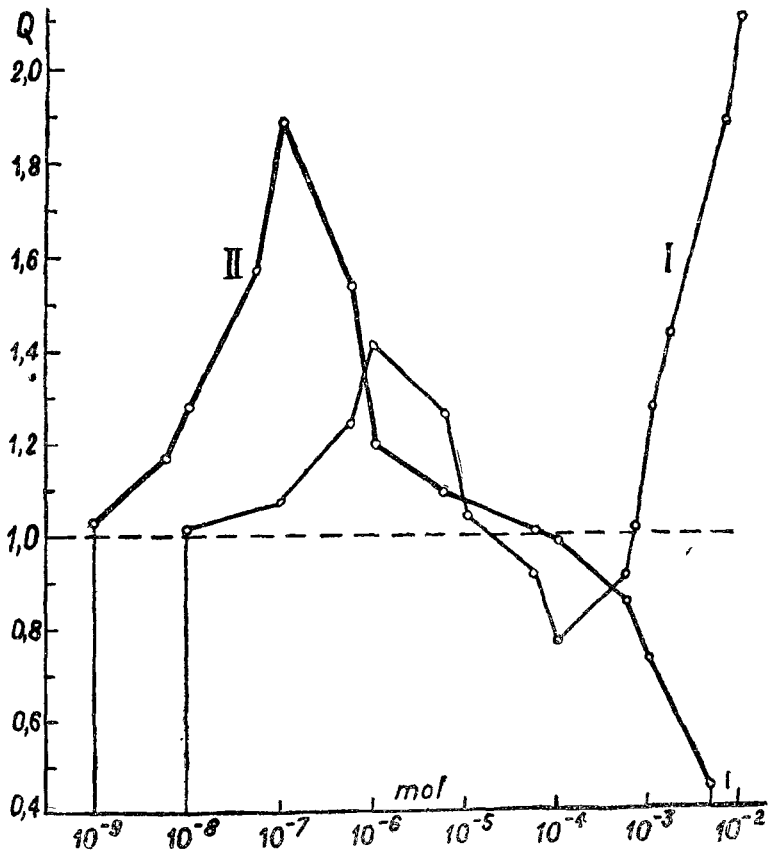
In the earlier stages of research with fusaric acid the main emphasis has been with reference to its effect on the water permeability of the plasma membrane (Bachmann, 1956, 1957) and on the water economy of the tomato plants (Gäumann, Kern, Schüepp & Obrist, 1959). The test objects used in the permeability studies were the epidermal cells of *Rhoeo discolor* (L'Her) and *Spirogyra nitida* (Dillwijn) Link. The host plant, tomato, was found unsuitable on technical grounds for these experiments and only a few experiments were conducted with its medullary cells. The measure of injury to the water permeability of plant protoplasts is expressed as a quotient of the time taken by cells for deplasmolysation after treatment with water divided by the time taken after treatment with the toxin concentrations. In the case of *Spirogyra* 0·8 molar sugar solution and in the case of *Rhoeo* 0·3 molar mannitol served as the plasmolytic substances.

$$Q = \frac{D_k}{D_t}$$

D_k = average time for deplasmolysation of the control in tap water
D_t = average time for deplasmolysation in toxin concentrations. Q values above 1 means an increase in the water permeability and below 1 means a lowering.

Fusaric acid begins to measurably impair the water permeability of the protoplasts of the epidermal cells of *Rhoeo discolor* at a low concentration of 10^{-9} molar and that of *Spirogyra nitida* at 10^{-8} molar. With increasing concentrations of the toxin i.e., between 10^{-7} and 10^{-6} molar, the water permeability is temporarily increased and the Q value is double that of the control in *Rhoeo* and one and a half times in *Spirogyra* (Text fig. 1). The medullary cells of tomato show initial response to fusaric acid only at a concentration of 10^{-7} molar and hence are less sensitive than the other two model objects. But for this, the injury pattern is similar to that in the other two plant species. Thus the initial increase in water permeability in all the three test objects follows a similar course. This shows that the physiological injury suffered by these protoplasts is not determined by the plant species.

With a further increase in toxin concentration the water permeability of the injured protoplasts returns to the normal value



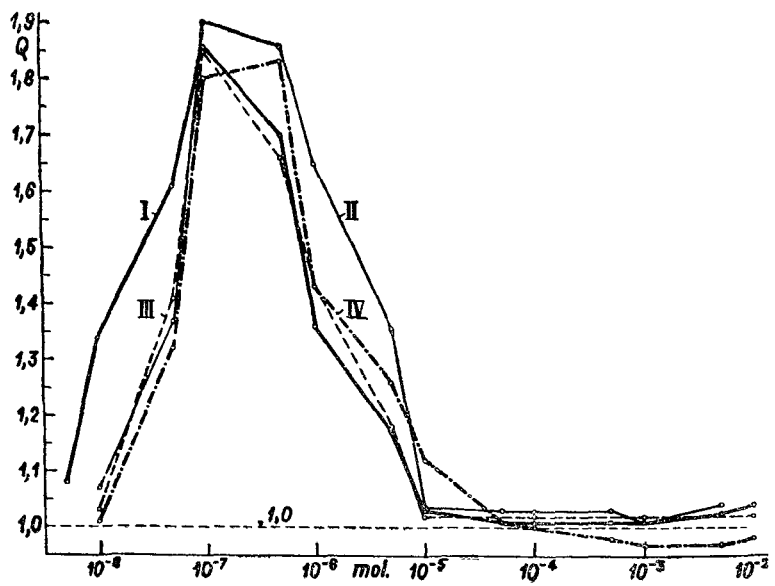
TEXT-FIG. 1. The effect of increasing concentrations of fusaric acid on the water permeability of protoplasts of· I = *Spirogyra*, II = *Rhoeo*. *Abscissae*: molar concentration of fusaric acid. *Ordinates*: degree and type of injury to water permeability expressed as Q-Value. (After Bachmann, 1956).

or, the protoplasts behave like controls in water. The Q value is 1 at the toxin concentration of about 10^{-5} — 10^{-4} molar (Fig. 1).

At still higher concentrations of the toxin a third decisive phase of injury sets in. Perhaps this phase of the injury plays a decisive role in pathogenesis. It is now known that this concentration of fusaric acid may be attained in cotton plants under the diseased conditions (Kalyanasundaram & Venkat Ram, 1956). In contrast to the earlier common behaviour by all the three test

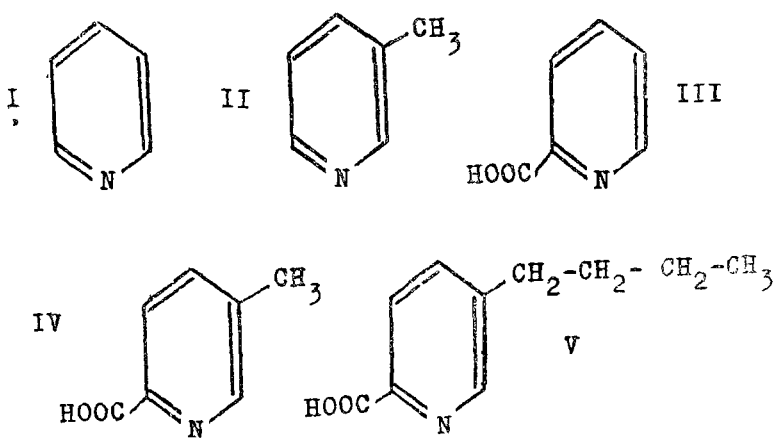
objects, this phase is determined by the type of plant protoplasts and hence the plant species. In *Spirogyra nitida* toxin concentrations above 10^{-5} molar induce an initial decrease in water permeability which is quickly reversed and results in permanent increase in permeability of the protoplasts. *Rhoeo* and medullary cells of tomato respond in a manner opposite to that of *Spirogyra*. The decrease in water permeability which sets in at toxin concentrations above 10^{-5} molar continues with increasing toxin dosage and a definitive "waterproofing" sets in. It looks as if the plasma membranes have coagulated.

Dehydrofusaric acid (5-n butylenepyridine-2-carboxylic acid) injures the water permeability of plant protoplasts i.e., of *Spirogyra* and *Rhoeo* in a manner similar to fusaric acid except that the slight "waterproofing" stage in *Spirogyra* seen in the second phase of the injury is absent here.



TEXT-FIG. 2 The influence of increasing concentrations of pyridine (curve I), α-picolinic acid (curve II), 3-methylpyridine (β-picoline; curve III), and 5-methylpyridine-2-carboxylic acid (methyl picolinic acid; curve IV) on the water-permeability of protoplasts of *Rhoeo discolor*. *Abscissae*: molar concentration of solutions *Ordinates*: degree and kind of impairment of water-permeability, expressed as Q-value (After Bachmann, 1956).

In this alternating injury to the water permeability of plant protoplasts by increasing concentrations of fusaric acid, two constituents of its molecule are involved; the pyridine ring and the n- butyl group in the β position. The first phase of injury caused by fusaric acid in all the three test objects studied, namely the transient increase in the water permeability, can be induced, in addition to the above toxin, by pyridine alone and twenty other derivatives of fusaric acid, whose sole common constituent is the pyridine ring (Text fig. 2). These are differentiated from one another only by their side chains. These substances at higher concentrations are not capable of producing that phase of the injury caused by fusaric acid concentrations above 10^{-5} molar i.e., the tight "waterproofing". Thus according to Bachmann (1956) the pyridine ring of the fusaric acid molecule controls the first phase of the injury viz., the transient increase in water permeability of the plasma membrane. The carboxyl group in the α position of the pyridine ring (α- picolinic acid), or the methyl group in the β position (β- picoline) or their combination giving rise to 5-methylpyridine-2- carboxylic acid (Text fig. 3, I, II, III & IV) are



TEXT-FIG. 3. I Pyridine; II. 3-methylpyridine (β-picoline); III. Pyridine-2-carboxylic acid (α-picolinic acid); IV. 5-methylpyridine-2-carboxylic acid (methyl picolinic acid); and V 5-n butylpyridine-2-carboyxlic acid (fusaric acid).

not toxic at concentrations above 10^{-5} molar. Hence, the specific injury to the plant protoplasts at higher toxin concentrations must, therefore, be determined by the n- butyl group in the β position. Thus, 3-n- butyl pyridine which differs from fusaric acid molecule

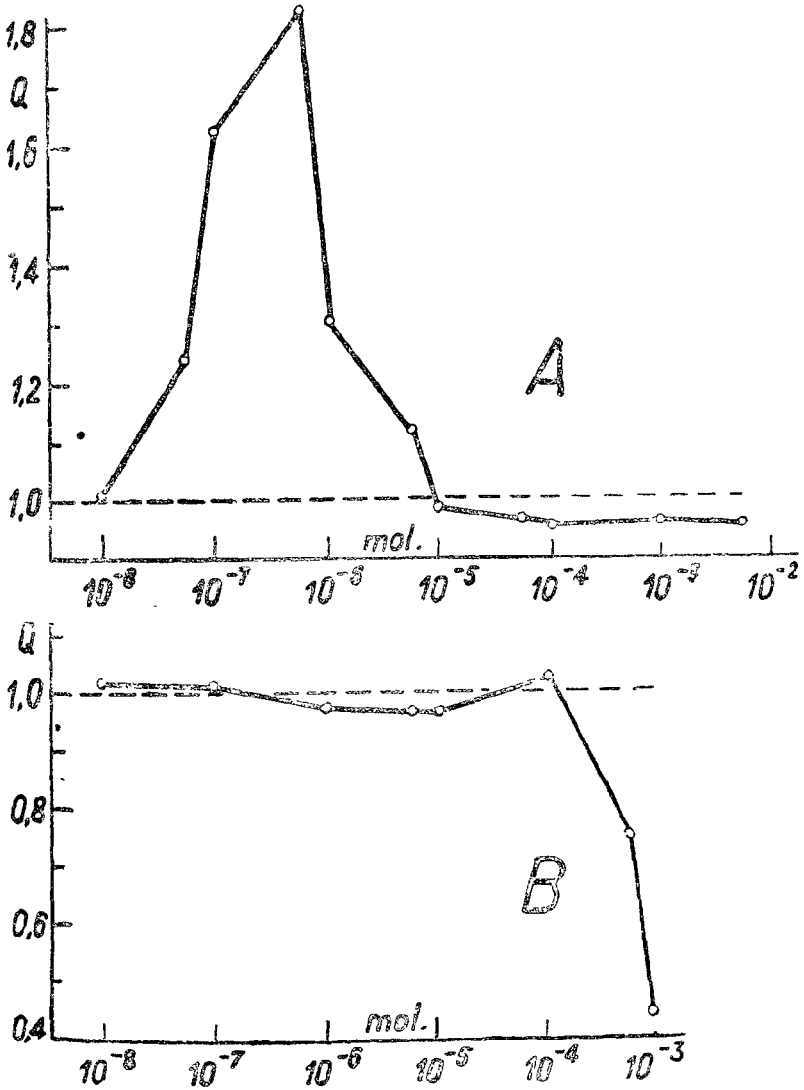
only in its lack of the carboxyl group in the α position produces the same effect as fusaric acid. In fact, a similar effect could be demonstrated with pyridine derivatives possessing the alkyl side chain. Homologous compounds lacking the side chain have no effect at higher concentrations.

Not only the effect on the permeability of plant protoplasts at higher concentrations of fusaric acid, is brought about by the aliphatic side chain, but the quantitative nature of the damage is determined by the length of this chain. The pathogenic action of this aliphatic side chain at first increases with the length of the chain. The significance of the carboxyl group in the fusaric acid molecule with reference to its toxicity is not yet clear.

Injury to the Host Ferment Systems

Two different processes are concerned in the intake of water by protoplasts. The familiar physical osmotic component, which obeys Fick's law of diffusion, and an active non-osmotic component that is supported by the energy metabolism of the living cells. Unlike the former, the non-osmotic water intake acts independently of the concentration gradient. The transport mechanism here is not by diffusion but through a system of transport molecules. The molecule to be taken is closely bound to a protein and carried along with it (Goldacre, 1952). This non-osmotic water intake acts at the expense of energy released by cell respiration. Conversely, impairment of cell respiration leads to a dysfunction of non-osmotic water intake. Hence impairment in water permeability brought about in toxigenic wilting could be due to the injury to an essential energy-releasing process which controls the water intake. It is possible to demonstrate this with simple plasmolysis experiments by suitably blocking the appropriate enzymes with specific poisons.

A few interesting experiments done by Bachmann (1956) are worth mentioning here. When epidermal cells of *Rhoeo*, in which the enzyme cytochrome oxidase is blocked previously by sodium azide, are subjected to increasing concentrations of fusaric acid, the water intake at lower toxin concentrations shows the marked rise characteristic of the pyridine ring. On the other hand, the curve showing the decrease in water permeability at toxin concentrations above 10^{-5} molar is completely absent. She concludes that the n-butyl group in the β position which is responsible for the action of the fusaric acid at higher concentrations is therefore



TEXT-FIG. 4. Impairment of the non-osmotic water intake of the protoplasts of *Rhoeo discolor* by increasing concentrations of fusaric acid, if, before treatment with fusaric acid, A) the cytochrome oxidase or B) oxidative phosphorylation in the *Rhoeo* protoplasts has been arrested. *Abscissae*: molar concentration of fusaric acid. *Ordinates*: degree and type of injury to the water-permeability, expressed as Q-value. (After Bachmann, 1956).

rendered inactive by the previous blocking of the enzyme cytochrome oxidase (Text fig. 4, A).

On the other hand, if one previously arrests oxidative phosphorylation in *Rhoeo* protoplasts with 2-4-dinitrophenol and subjects them to increasing fusaric acid concentrations, the curve showing increased permeability conditioned by the pyridine ring is absent at lower fusaric acid concentrations (Text fig. 4, B). At concentrations above 10^{-4} molar the characteristic decrease in water permeability brought about by the alkyl side chain sets in. By this Bachmann (1956) could demonstrate that the pyridine ring of the fusaric acid molecule has been inactivated by the previous blocking of oxidative phosphorylation. Thus the pyridine ring in some way disturbs the oxidative phosphorylation.

Fusaric acid thus impairs the energy metabolism and in consequence the non-osmotic water intake of plant protoplasts by two different mechanisms at lower and higher concentrations.

Fusaric acid ethyl ester causes injury to the water permeability of plant protoplasts in a manner similar to that of fusaric acid, although, it cannot form metal chelates. Hence, it is unlikely that this injury caused by fusaric acid is due to chelation with heavy metals. In conclusion Bachmann says "How fusaric acid interferes with different enzyme systems is not yet clear."

Mode of Action of Fusaric acid on Micro-organisms

The answer to the above question has been forthcoming recently with the work done on the effect of fusaric acid on certain micro-organisms. In the classical sense of the term, fusaric acid is a weak antibiotic. It inhibits the growth of bacteria, yeasts and the yeast-like fungus *Candida vulgaris* Auct.. Ever since the discovery (Woods, 1940) that sulfanilamide acts in bacteria by competitively replacing p-aminobenzoic acid, one of the essential metabolites of the bacterium, evidence is growing that many drugs, antibiotics and toxins affect the susceptible organisms by antimetabolite action. There are many examples of competitive antagonism to an essential metabolite in the field of antibiotics and antivitamins. However, very few cases have been demonstrated in the field of phytopathologically interesting toxins. That such possibilities exist is shown in the wildfire disease of tobacco which shall be dealt with later.

Although the action of fusaric acid is manifold, we may reasonably expect that some of the injuries caused are basic and more fundamental in nature, and therefore common to all organs and organisms susceptible to fusaric acid. If this is so, the other host specific and tissue specific injuries play an additive role in the clinical picture, or, they superimpose on the ground effect. With this in view, we asked ourselves the following questions:

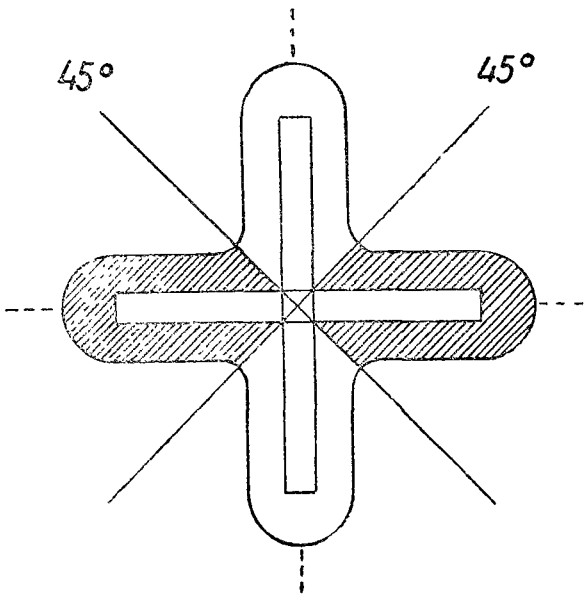
Is the toxicity of fusaric acid fully or in part attributable to its antagonism of some essential metabolite of the susceptible organisms or tissues? If so, is it possible to remove its toxicity to susceptible organisms and organs by administering the hypothetical metabolite?

Pyridine derivatives and certain of the vitamins of the B group which are analogous in structure to fusaric acid were screened for their capacity to nullify the toxicity of fusaric acid to micro-organisms. However, the results were without success (Kalyanasundaram, 1960; Braun, 1960). In spite of this earlier failure, it is clear now that the toxicity of fusaric acid to bacterial species and certain fungi stems from the fact that it interferes with a metabolite essential for the growth of these organisms (Kalyanasundaram, 1960). This demonstration became possible because it was observed that the sensitivity of *Bacillus subtilis* Cohn, and *Escherichia coli* (Mig.) Cast., to this antibiotic is increased greatly in a synthetic medium than in a medium with beef or yeast extract. Subsequent work led to the demonstration of the presence of a substance in yeast extract that could reverse the toxicity of fusaric acid to these test organisms, and this reversal appears to be competitive.

The antagonist of fusaric acid present in yeast extract could be separated by adsorption on frankonite and subsequent elutions. In two-dimensional chromatograms the substance could be located as a single spot with a consistent R_f value, in specific solvent systems. Results obtained by paper chromatography and adsorption on ion exchange resins point to the fact that the antagonist of fusaric acid has acidic character.

The competitive nature of the reversal of the toxicity of fusaric acid by the factor from yeast extract was demonstrated by the cross strip technique developed by Bonifas (1952) and Juillard (1957). Two filter paper strips 5 mm broad and 6 cm long, are

dipped in the solution of the metabolite and the anti-metabolite respectively, and are dried between blotting paper. The two strips are laid at right angles on the agar surface in a Petri dish containing the test organism. If the two substances diffuse identically, they produce an area of diffusion taking the shape of a cross. This may be possible when the two substances are structurally similar and of a similar molecular size. Where the two substances meet and overlap there are points where the ratio of concentration of the two substances are the same. These points lie on two lines running at an angle of 45° to the axes (Text fig. 5).



TEXT-FIG. 5. Diagram of competitive antagonism with the cross strip technique. The horizontal strip contains the antimetabolite and the vertical one the metabolite. (After Juillard, 1957).

At a particular concentration of the metabolite and the anti-metabolite, these two lines pass through the coaxial point. At this concentration the ratio of the amount of the metabolite to anti-metabolite gives the inhibition index (Juillard, 1957).

With *Candida vulgaris* as the test organism and using the yeast extract factor and fusaric acid it was possible to get a pic-

ture similar to that theoretically expected (Plate 1, Fig. 4). The inhibition index could not be precisely obtained as the factor contained an unknown number of impurities. When the inhibition index is constant over a wide range of concentrations the antagonism is said to be competitive (Woolley, 1952). In the cross strip technique, by varying the concentration of one of the substances (factor), the border-line between inhibition and growth zone undergoes a parallel shift. Only at one concentration, however, does the line of 45° pass through the coaxial point. Thus we have evidence that fusaric acid inhibits the growth of *Candida vulgaris* by competitively inhibiting the action of a substance essential for its metabolism. A similar picture was obtained in the tests conducted with *Bacillus subtilis* Cohn, and the fungus *Ustilago sphaerogena* Burr. ex Ellis *et* Everh.. However, with *Escherichia coli* (Mig.) Cast. and *Saccharomyces cerevisiae* Hans., the yeast factor and fusaric acid showed non-competitive antagonism (Kalyanasundaram, 1960).

Studies made on the effect of fusaric acid and pyridine derivatives on the water permeability of plant protoplasts show that the specific injury caused by fusaric acid at concentrations above 5×10^{-4} molar is, to a large extent, due to the length of the aliphatic side chain of the pyridine ring. It was therefore of interest to study the activity of the yeast factor against structural analogues of fusaric acid with differing lengths of the aliphatic side chain.

TABLE 2

Diameter of the inhibition zone caused by different pyridine derivatives on test plates of *Candida vulgaris*. Concentration used 5×10^{-2} molar. (After Kalyanasundaram, 1960).

Fusaric acid	..	21·5 mm
Dehydrofusaric acid	..	20·5 mm
Ethyl picolinic acid	..	10·0 mm
Methyl picolinic acid	..	7·5 mm
Picolinic acid	..	0·0 mm
3 n-butyl pyridine	..	0·0 mm

Experiments were conducted to study the toxicity of the following substances with a decreasing length of the aliphatic side

chain, on the test organism *Candida vulgaris*: fusaric acid (5-n butylpyridine-2-carboxylic acid), dehydrofusaric acid (5-n butylenepyridine-2-carboxylic acid), 5-ethyl-picolinic acid (5-ethylpyridine-2-carboxylic acid), 5-methyl-picolinic acid (5-methyl-pyridine-2-carboxylic acid), and picolinic acid (pyridine-2-carboxylic acid). In addition one pyridine derivative was used which did not contain a carboxyl group, namely, 3-n butylpyridine. It was observed that picolinic acid and 3-n butylpyridine showed no toxicity. But with all the other compounds tested, greater toxicity was observed with an increase in the length of the aliphatic side chain (Table 2). Thus, given the basic structure of α-picolinic acid, the length of the alkyl side chain of the analogues determine the magnitude of their toxicity.

A somewhat similar situation occurs with the vitamin thiamine and its structural analogues. The vitamin has a methyl group at position 2 of the pyrimidine ring. The corresponding ethyl analogue has only 10% of the original vitamin activity. The butyl analogue, however, is an antimetabolite (Woolley, 1952). In contrast to this situation even complete removal of the butyl group at position 5 of the pyridine ring of the fusaric acid molecule leading to picolinic acid did not give rise to the metabolite.

The toxicity of the pyridine compounds were competitively reversed by the factor from yeast extract. However, with the decreasing toxicity of the ethyl and methyl analogues the activity of the factor increased.

In fact, the toxicity of fusaric acid to cut shoots of tomato plants could be partly removed by this anti-fusaric acid factor isolated from yeast extract. About this I shall deal with in the second part of this lecture i.e., physiology of defence reactions.

We have already seen that the specific injury to the water permeability of plant protoplasts, brought about by fusaric acid at concentrations above 10^{-5} molar, is determined by the n-butyl group in the β position and that the fusaric acid molecule somehow interferes with the working of the enzyme cytochrome oxidase. We are dealing with an identical situation in our studies with bacteria and fungi as here also fusaric acid is toxic to these micro-organisms only at concentrations above 10^{-5} molar. As before, here again the magnitude of the toxicity is determined by the length of the alkyl side chain of the pyridine ring. It is this

specific toxicity that is reversed competitively by the factor isolated from yeast extract. On the basis of the existing definition of competitive antagonism we might conjecture that fusaric acid interferes with the functioning of an essential metabolite which is most probably closely associated with the enzyme cytochrome oxidase.

During the course of, and immediately after these experiments were conducted by the author, Braun, working on the products of conversion of fusaric acid by microorganisms, got certain interesting results which have since been published (1960). The fact that the metabolite which reverses the toxicity of fusaric acid was found in the culture filtrate of *Fusarium lycopersici* led us to believe that a substance closely related to fusaric acid and capable of antagonising the toxin is formed by the fungus in the biosynthesis of fusaric acid, or conversely, in the conversion of fusaric acid. The products formed by the conversion of fusaric acid by fungi, and a series of synthetic substances very near in their structure to fusaric acid were investigated by Braun but without success.

As opposed to these, Braun (1960) found that ferrioxamine, an organic substance of microbial origin with a moiety of iron in it, could remove the toxicity of fusaric acid to the test organism *Candida vulgaris,* with an inhibition index of 970. This was demonstrated with the other test organisms like *Saccharomyces cerevisiae* and *Ustilago sphaerogena* which were earlier used in the studies on the antagonist of fusaric acid. Braun reports that he could achieve this reversal of toxicity of fusaric acid with certain other compounds containing iron. It was found that a gram atom of iron removes the action of even 1000 molecules of fusaric acid. In such a relationship there is no possibility of direct chelation because a single atom of iron could not possibly bind 1000 molecules of fusaric acid. The finding could be explained only by the fact that fusaric acid specifically attacks the iron metabolism of the test organism (Braun, 1960). Although the antagonism appeared competitive as demonstrated by the cross strip technique, as the experiments could not be conducted on a wider range of concentrations and as the two antagonists are not structurally similar, it could not be called as competitive on the basis of the existing definition (Braun, 1960).

That the toxicity of fusaric acid to microorganisms could be reversed by an unidentified factor from yeast extract, which is a

substance essential for the metabolism of certain bacteria and fungi and probably related to the structure of fusaric acid, as well as by iron, brings in certain new points of importance in the above antagonism. Such cases of antagonism are known. For example the inhibitory effect of pyridine-β-sulfamide on *Streptobacterium plantarum* Orla-Jensen was reversed by nicotinic acid, nicotinamide as well as by iron (Möller and Birkofer, 1942). Thus, the mode of action of this antibiotic cannot be explained purely on the basis of its blocking the action of the vitamin nicotinic acid but is also due to a blockage of the needed iron.

Toxin from a Parasite with Short Range Action

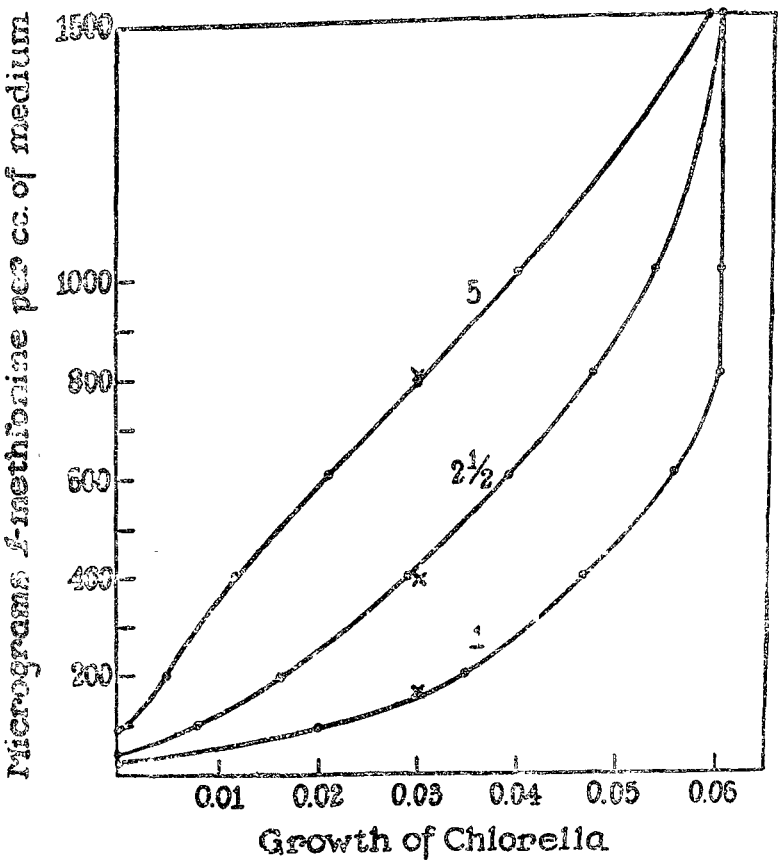
All the plant pathogenic fungi which form fusaric acid produce, besides this and the usual non-specific enzymes, a number of specific toxins and related substances. As the pathogenic action of fusaric acid in the natural diseased state is conditioned by complex factors, the symptoms of fusaric acid injury and disease symptoms are not the same. The situation here is therefore more complicated than it is, for example, in the wildfire disease produced by *Pseudomonas tabaci* (Wolf & Foster) Stev., in tobacco. This toxin is the only decisive toxin formed by this pathogen and it therefore acts by itself within the host and hence, symptoms produced by the toxin and the pathogen are similar. The physiological and chemical studies involved in the study of this toxin are a great step forward in plant pathological research.

The bacterial leaf spot disease of tobacco, first described by Wolf and Foster in 1917 in America, is now found to occur in most of the tobacco growing regions. Because of the suddenness of appearance, the rapidity of spread and the severe nature of the disease in the field the term 'wildfire' has been applied to this disease. Johnson and Murwin (1925) first demonstrated that the culture filtrate of the pathogen could produce the lesions characteristic of the disease on the host plants, and that the toxin is not host specific although the pathogen is.

In studying the mode of action of this toxin Braun (1950) has used the unicellular alga *Chlorella vulgaris*. He demonstrated that the toxin inhibited the growth of this organism. However, when yeast extract or liver extract is added to the toxin there is a reversal of its deleterious effect. This suggested that the toxin interfered with the functioning of some essential metabolite necessary for the growth of this alga. Yeast or liver extract provided

this metabolite when added on to the culture, externally. A further investigation showed that l-methionine, one of the constituents of the above extracts, reversed the action of this toxin on this alga.

One of the probable pathways of methionine synthesis in bacteria and fungi is as follows: cysteine—cystathionine—homocysteine—methionine. The three precursors when tested separately were found to be incapable of overcoming the effect of this toxin. The immediate precursor, homocysteine, forms methio-



TEXT-FIG. 6. Curves showing growth of *Chlorella vulgaris* with three different concentrations of the wildfire toxin (1, 2½ and 5) against a range of concentrations of l-methionine; note that at half growth (x) the ratio between toxin and methionine is constant. (After Braun, 1955).

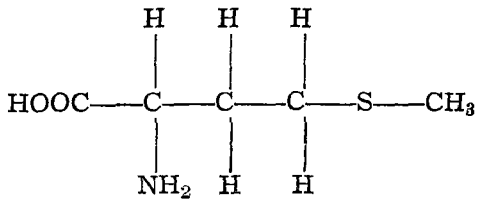
nine by transmethylation. If the synthesis of methionine in *Chlorella* follows a pattern similar to that described for other organisms, then the toxin might exert its biological effect either by blocking the methylation of homocysteine or by interfering with the utilization of methionine that is normally synthesised by *Chlorella*. To test the first possibility homocysteine was administered with choline or betaine, two good methylating agents, in the presence or absence of *p*-aminobenzoic acid, to the test organism subjected to the wildfire toxin. The toxicity was, however, not reversed. Since transmethylation is a complex phenomenon we are not in a position to conclude that the toxin does not interfere with the methylation of homocysteine. However, it was proved by Braun (1955) that the wildfire toxin competes with methionine for active centres on the enzyme that normally combines with or acts on methionine. This could be demonstrated by the competitive nature of the antagonism between methionine and the wildfire toxin with the test organism *Candida vulgaris* (Text fig. 6).

A similar phenomenon could not be demonstrated with tobacco leaves treated with the toxin and methionine. These results suggested either that the mechanism of action of the toxin is different in the two plant species, or that the action was the same but it was difficult to demonstrate in the higher plants, due to certain practical difficulties. Nevertheless, one could conclude that the wildfire toxin is a structural analogue of methionine and its biological activity is due to its behaviour as an antimetabolite.

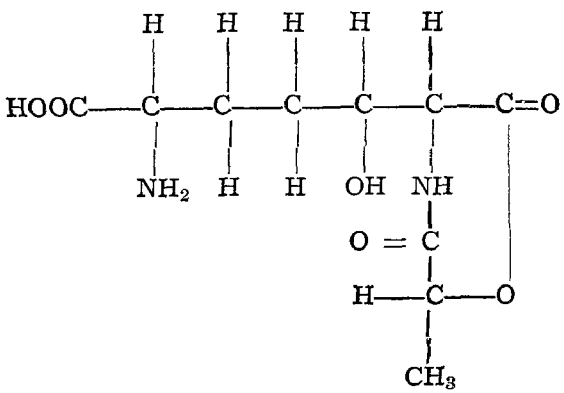
The wildfire toxin has been found to be a derivative of a new α-amino acid. (Woolley, Schaffner & Braun, 1952). Complete hydrolysis of the pure toxin with strong HCl yields lactic acid and large amounts of this new amino acid which has been found to be α, ε-diamino-β-hydroxy pimelic acid. The name tabtoxinine has been applied to this substance. The amino acid is the second member of a new type of α-amino acids (sulphur-free diaminodicarboxylic acid) found to occur naturally. Tabtoxinine has been found to be biologically inactive in so far as its ability either to elicit the formation of or to reverse the chlorotic effect of wildfire halos in a tobacco leaf.

The wildfire toxin has been found (Woolley, Schaffner and Braun, 1955) most probably to be the lactone of α-lactylamino-β-hydroxy-ε-amino pimelic acid. The toxin is biologically a very

active substance and 0·05 gamma gives rise to a typical chlrotic lesion when introduced in the tobacco leaf.



METHIONINE



WILDFIRE TOXIN

The relationship of methionine and the toxin can be seen clearly by comparing the structure of these compounds. In the toxin the sulphur atom of methionine has been replaced by two carbon atoms. A change such as this commonly converts metabolites into antimetabolites. One of the carbon atoms of the toxin bears the oxygen atom and the other bears the nitrogen atom. In addition, in the toxin, the oxygen, and nitrogen bonds have been reduced, and the methyl group has been oxidised to a lactone grouping. From previous studies it appears that the chemically high reactive group, lactone, present in the toxin, may make it a potent antimetabolite. This lactone most probably binds the toxin by a covalent bond to the site in the plant cell that is normally occupied by methionine (Braun, 1955).

I have presented above the mode of action of two toxins; fusaric acid, an organic acid of fungal origin, *pleiotropic* in action and systemic in nature; and wildfire toxin, an amino acid derivative of bacterial origin, highly specific in action and localised in nature.

II. PHYSIOLOGY OF DEFENCE REACTIONS

We have already dealt with the disease from the parasite's point of view; especially with reference to the toxins which evoke the pathological reactions of the host. This ability of the parasite to evoke disease is called pathogenicity. It is a measure of its parasitic efficiency. Those which lack this capacity are said to be apathogenic.

From the side of the host, equally strong and virulent reactions are produced to fight the parasite and the disease it causes. By defence reaction we understand a vital process initiated in a host by a pathogenic agent and directed more or less against that agent itself (Gäumann, 1950). These reactions may be either autonomous or induced depending on their genesis. Three types are recognised upto now depending on the object of these reactions.

When the host reactions are directed solely against the pathogen and are meant to contain it, prevent its spread, or weaken it, or even destroy it we call them as anti-infectional defence reactions. Perhaps, it will not be out of place if I describe here certain attributes of the host and the parasite which are essential prerequisites in any successful pathogenesis. The first is "affinity" between the host and the parasite. On the side of the parasite, it is its attraction towards a specific host and on the side of the host it is its "inclination" to serve as host (Gäumann, 1950). Granting that these conditions are satisfied, the parasite must possess the capacity to invade and generalise within the host. It must inhabit and feed freely on the host. The host on its part offers resistance to the pathogen. The capacity of the parasite to overcome this resistance is called as "aggressiveness". Hence, *ceteris paribus* the first type of defence reactions is directed against this quality of the parasite.

The condition of the host is not necessarily hopeless if this line of defence falls. It still has the possibility to direct its reactions against the ultimate stage in pathogenesis i.e., toxin production. These are the antitoxic defence reactions.

A third type of defence reactions goes under the name of 'induced tolerance'. This is directed neither against the parasite nor its toxic products. Here, a desensitization occurs in the plant itself, and it 'tolerates' the pathogen and its metabolic products without the manifest disease.

In actual life it is difficult to demarcate these functional types, and also it is not to be understood that they act independently of each other, or again, in a stepwise fashion to defend against the disease and its agent. In life, it is their totality that is effective. However, for the sake of convenience in presentation these three types will be discussed below separately with certain known examples.

Anti-Infectional Defence Reactions

These reactions are mainly directed against the parasite and in spite of their name are unable to prevent infection. Infection therefore 'takes' and the reactions succeed in so far as they confine the pathogen to specific tissues. Perhaps the pathogen may eventually be thrown out completely.

Thus, in quite a number of parasitic infections of plants, the invader is localised in particular tissues or kept at a particular region by the innate specific characters of the host. This spontaneous reaction of the host is termed as autonomous defence reaction. This results in chronic disease and here the pathogen is kept biologically weak or subdued in the periphery, or in localised areas of the host by its biochemical defence. The phenomena of digestion exhibited *in vivo* in root nodules of Leguminoseae and embryos of orchids, infected by the nodule bacteria and mycorrhizal fungi respectively, are some of the examples.

In a typical disease, the balance of forces operating between the host and parasite is shifted in favour of the parasite and hence pathogenesis sets in. In the above mentioned cases a fair degree of balance has been achieved between the host and the parasite and the latter is unable to evoke the typical disease symptoms. More than that, the host is even able to incite certain favourable responses from the parasite. This could be easily understood, if only we can visualise that the aggressiveness and pathogenic potential of the root nodule bacteria or the mycorrhizal fungi, as the case may be, have been knocked out by the host defence reactions. The apathogenic or the weakened parasite is tolerated by the host, as its presence bestows certain benefits to the host.

Hence, technically we are in our limits to call these as infectious diseases although, the term 'mutualistic symbiosis' is generally used to denote this secondary beneficial effect which the parasite confers on the host in response to infection.

While the above examples serve to show the autonomous anti-infectional defence reactions, I shall be dealing here with the more recent work that points to an induced anti-infectional defence seen in the orchids *vis a vis* mycorrhizal fungi.

When a pathogen attacks a host, among the manifold reactions it evokes in the host a few are beneficial to the latter. For example, the parasite stimulates or activates in the host a readiness for defence which was latent or dormant till then, with the result that it can now do things of which it was formerly incapable. The newly appearing defence reactions are called as induced anti-infectional defence reactions.

The infection produces a sensitization, a heightened disposition of the tissues or the whole organism to the pathogen. This sensitization can be brought about by the parasite itself, or its metabolic products. In the case of orchids to be discussed below the second type of sensitization i.e., by the metabolic products of the pathogen, plays a role.

In man sensitization by the parasite itself occurs spontaneously through the diseases of the childhood like measles, whooping cough etc.. Based on this principle is the prophylactic vaccination against small-pox. In medicine prophylactic diphtheria and tetanus inoculations are based on the second possibility i.e., sensitization by the metabolic products of the pathogen.

Induced chemical defence in orchids

The roots of ceratin orchids are regularly infected by mycorrhizal fungi, but seldom their bulbs. However, if they were to be infected, the fungus is then contained in the peripheral zone. The investigations upto now show that their full or partial immunity is due to antibiotic activity of probably two substances, one present in the bulbs prior to infection and the other formed after infection. The latter substance is formed in response to infection and has been demonstrated to be a product of induced defence reactions.

The presence of an antifungal substance in the bulbs of orchid infected with *Rhizoctonia repens* Bern. was first demonstrated by

Bernard (1911) and Nobécourt (1928) with *Loroglossum hircinum* (L.) Rich. growing in the Jura mountains of Southern France.

If this fungus is grown on an agar medium and an aseptic piece of sufficient size of the orchid bulb is cut out and placed on the seeded medium, the fungal growth is inhibited around the bulb. If the bulbs are killed prior to plating, either by chloroform or low temperature (— 10°C.) the fungus is not checked but grows vigorously through the bulb. Hence it was concluded by these workers that the antifungal substance is produced by the live orchid bulb as a reaction to the fungal infection. About twenty years later a similar phenomenon was observed in the tubers of *Orchis militaris* L. infected by *Rhizoctonia repens* Bern. (Gäumann & Jaag, 1945). Further work was undertaken by Gäumann and his co-workers to isolate this substance and also to understand its nature.

Is there a natural chemical protection in the orchid bulbs?

Fresh bulbs of *Orchis militaris* L. which are not wounded and which do not have any local infection, were found to contain regularly, on chemical analysis, the substance cumarin ($C_7H_6O_2$, mol. wt. 146) in the order of 0·05 to 0·4% of their fresh weight. This is about a concentration of 4×10^{-4} molar in the cell sap of these bulbs. The antibiotic activity of this preformed substance is low and its spectrum very narrow. It therefore appears that the spontaneous chemical defence in the tissues of the orchid tubers is low. As opposed to the potato tubers, these bulbs also do not have much of a mechanical protection. Yet, they are rather well protected against infections. Hence, the mechanism of defence lies in some other sphere and it is here that the induced chemical defence reaction is supposed to play a role. It was possible to isolate this antifungal substance and describe its biological and chemical character after twelve years of work by Gäumann and his co-workers. This substance is orchinol, with the empirical formula of $C_{16}H_{16}O_3$, and it is a cryptophenol i.e., a phenol whose OH group does not react in the usual way.

Production of orchinol and its identification

The bulbs of *Orchis militaris* L. were successively dug out in autumn and winter (August-February) and, after thorough washing, were surface sterilised with mercuric chloride or propylene oxide or both, and finally washed with sterile water. Under sterile conditions they were cut in the middle to give

cubes of about 2 cm which were filled in sterile glaxo flasks. They were subsequently inoculated with a spore suspension of the fungus *Rhizoctonia repens*. Incubation was done at 26°C for 10 to 14 days, by which time the fungus had made a felt-like growth on the bulbs. These were taken up for further extraction and chemical analysis (Gäumann & Kern, 1959).

The bulbs of *Orchis militaris* were found to contain orchinol and three other fractions, of which one has been identified as *p*-hydroxy-benzylalcohol. Compared to orchinol, the other fractions have practically no biological activity against the test organisms.

In paper chromatograms with methanol water solvent (1:1) and at a temperature of 26°C orchinol has an R_f value of 0·36. It can be detected on the paper by the following characters:

(a) It reacts with dichloroquinone chloramide to give a green grey spot. The lowest detectable amount is 10γ.

(b) It shows characteristic violet fluorescence in ultra violet light. The lowest detectable amount is 1γ.

(c) It has green luminiscence after being subjected to ultra violet light.

The quantitative estimation is based on its fluorescence in u.v. light. It is also possible to estimate it on the basis of its absorption spectrum in u.v. rays with the peak at 280 μ. About 20 γ of the substance can be detected by this method. Colorimetric method of estimation is also possible based on the colour reaction with diamide and the reading taken at 680 μ in a Spectrophotometer.

Using the above outlined methods it could be demonstrated that orchinol was present in the infected bulbs of *Orchis militaris* but not in the healthy and fresh ones. These results do not conclusively show that orchinol is a product formed by the bulbs in response to infection. One could as well imagine that orchinol could have been formed by processes of fermentation of the fungus, *Rhizoctonia repens*, in the orchid bulbs without the latter having anything to do with the reaction. In that event the mycorrhizal fungus will be playing the same role in the bulbs of *Orchis militaris* as the yeasts in the alcoholic fermentation of overripe grapes. But that this is not the case is obvious from the following experiment. Bulbs killed by heat or cold and infected by *Rhizoctonia repens*, or, fresh uninfected bulbs do not contain any orchinol. On

the contrary bulbs activated by *Rhizoctonia repens* contain this substance (Table 3). Thus, orchinol is not formed or released from substances already present in the host by the fungus *Rhizoctonia repens*, but is a substance newly formed by the living bulbs as a result of infection by the mycorrhizal fungus.

TABLE 3

Orchinol content of the bulbs of *Orchis militaris* L. subjected to different treatments.

(After Gäumann & Kern, 1959)

Treatments	Quantity of bulbs used g	Orchinol content	
		% fresh weight	Approximate concentration in the cell sap in molarity
Direct extraction	1000	0	—
	930	0	—
	570	0	—
Extracted after infection with *Rhizoctonia repens*	7750	0·13	$0{\cdot}6 \times 10^{-3}$
	9580	0·14	$0{\cdot}6 \times 10^{-3}$
	220	0·25	$1{\cdot}1 \times 10^{-3}$
	8276	0·25	$1{\cdot}1 \times 10^{-3}$
	10000	0·29	$1{\cdot}3 \times 10^{-3}$
Killed and infected			
Heat	330	0	—
Cold (-25°C for 48 hrs.)	425	0	—

There are a few other substances known in plant pathology which seem to be formed in plants as a result of induced defence reaction, but are only apparently so. This type of reaction is to be distinguished from real induced defence reaction i.e., the production of orchinol. For example, allyl mustard oil occurs in cruciferous plants as a glucoside combination which is non-toxic to fungi. However, if the enzyme myrosin is released as a result of wound—this invariably happens when a fungus penetrates to infect—which splits the mustard oil from the glucoside, the released free mustard oil is highly toxic to the parasite. So here, the

fungus only incidentally helps in the release of the antifungal substance by the injury it causes, purely a side effect. But orchinol appears to be formed newly by the bulb and is not present in any combination in the bulb prior to infection.

The capacity to synthesise orchinol as a result of infection by the mycorrhizal fungus *Rhizoctonia repens* is not the sole prerogative of the bulbs alone, but the roots and stem are also known to possess this capacity. Only, the quantity synthesised is too low to have any biological significance. In the bulbs, once synthesised, it persists for months. Thus the bulbs, in which the production of orchinol is induced as a result of initial infection, are protected for months against further infection. This chemical resistance reaction therefore leads to acquired immunity.

Host spectrum for orchinol formation

Twenty-four species of orchids from Central and Southern Europe were tested for their induced chemical defence against a strain of the mycorrhizal fungus *Rhizoctonia repens* Bern. isolated from *Orchis militaris*. While 21 of the species tested developed *in vitro* characteristic zones of inhibition against the fungus (similar to that in Fig. 3 of Plate 1) three of them did not show any reaction and the fungus could grow over the bulbs (similar to that in Fig. 2, Plate 1). Thus in *Ophrys arachnites* (Scop.) Murray, *Orchis maculata* L. and *Plantanthera bifolia* (L.) Rich. the fungus could not set in motion the process of induced defence reaction. In the species showing positive reaction, orchinol could be shown to be present in different amounts in 18 of the host species, while in the three other species the zone of inhibition of the fungus is caused by some other substance in the place of orchinol. (Gäumann, Nüesch and Rimpau, 1960).

Of the three species of orchids that showed negative results, *Ophrys arachnites* and *Orchis maculata* (*Plantanthera bifolia* has not yet been tested due to certain practical difficulties) could be shown to develop characteristic zones of inhibition against their own specific mycorrhizal partners. Here also the substance responsible for the inhibition of fungal growth is not orchinol.

Thus it could be demonstrated that all the species of orchids tested possess the capacity for anti-infectional defence against specific mycorrhizal fungi. Perhaps this is a phenomenon common to all orchids.

The spectrum of microorganisms inducing orchinol formation

About 19 species of mycorrhizal fungi isolated from various species of European orchids were tested against the bulbs of *Orchis militaris* and all of them were able to induce the production of an antifungal substance which inhibited their growth. In the majority of cases the substance has been identified as orchinol. Most of these were species of *Rhizoctonia* and *Orcheomyces*. Only about four species tested were not able to induce this reaction and they were found to be strains isolated from exotic orchids. Most probably they had never encountered the host tissue of *Orchis militaris* before and hence they were unable to incite the reaction in this uncongenial host.

Rhizoctonia solani Kühn, a potential mycorrhizal partner of the European orchids *Orchis purpurella* T et T. A. Steph. and *Orchis ericetorum*, and isolated from one of them, is unable to induce the production of orchinol in the bulbs of *Orchis militaris*. In test plates the fungus is not inhibited by the tubers of *Orchis militaris* (Plate 1, Fig. 2).

Among the many fungi that are known to be soil inhabitants, twenty-four species were tested representing the genera *Mucor, Pythium, Rhizopus, Aspergillus, Botrytis, Cladosporium, Didymella, Fusarium, Neurospora, Trichoderma* and *Rhizoctonia*. None was able to set in motion in the bulbs of orchids the reaction for the formation of orchinol or related substances.

As opposed to this, some of the soil living bacteria, which are saprophytic or semi-parasitic, were able to induce the production of orchinol in the bulbs of *Orchis militaris*. Thus, *Erwinia carnegieana* Lightle *et al.*, *Erwinia nimipressuralis* Carter, and *Pseudomonas putida* (Trev.) Mig. which are regularly associated with the bulbs of *Orchis militaris* were able to synthesise orchinol. The other soil inhabiting bacteria like *Bacillus megatherium* de By. and *Bacillus subtilis* Cohn do not have this capacity. Thus the mycorrhizal fungi and those bacteria which are in some way associated with the orchid bulbs were able to set in motion the chain reaction leading to the synthesis of orchinol, while the unspecific saprophytic or semi-parasitic soil fungi and bacteria were unable to do so.

The spectrum of action of orchinol

Of the 19 mycorrizal fungi tested, 17 were inhibited by orchinol. The majority of these orchinol-sensitive fungi thus set in

motion, on infection, in the bulbs, reactions leading to the synthesis of this substance which in turn prevents the spread of these fungi themselves. The only mycorrhizal fungus which does not induce the synthesis of, and also is not sensitive to orchinol is *Rhizoctonia floccosa* Burg. from *Myrmechis gracilis*. *Rhizoctonia anomala* Burg. from *Orchis maculata* L. can induce the synthesis of orchinol in the bulbs of *Orchis militaris* but is not inhibited in its growth by orchinol, while *Rhizoctonia mucoroides* Bern. from the orchid *Phalaenopsis amabilis* and from *Vrijdagzynea albida* is orchinol-sensitive but is unable to induce the synthesise of the same in the host *Orchis militaris*.

The majority of the unspecific soil-living fungi which in their earlier tests for inducing orchinol production gave negative results were however, found to be sensitive to orchinol. As opposed to this, the soil living bacteria were not orchinol-sensitive. Thus some of these bacterial species induce the synthesis of orchinol, but yet are not sensitive to it.

The activity spectrum of orchinol is therefore relatively wide and unspecific. The first infection which initiates the production of this substance protects the host tissue not only against homologous reinfection but also produces a polyvalent protection against a whole series of parasites and saprophytes capable of secondary infection. In the classical sense of the term, orchinol is a weak antibiotic. It is capable of antifungal or antibacterial activity mostly at concentrations of 10^{-4} to 10^{-3} molar. Nevertheless, it is able to perform its task in the bulb tissues where the concentration of this antibiotic is enough to prevent the growth of the fungus. It must be pointed out that the concentration of orchinol encountered in these tissues is definitely much higher than the concentrations in which antibiotics are used in therapy in medicine.

Reaction time for the production of orchinol

In Table 4 is given the orchinol content of a piece of the bulb of *Orchis militaris* brought in contact with the mycorrhizal partner *Rhizoctonia repens* after an induction period of 12, 24 and 48 hours. Traces of orchinol can be detected at the end of 36 hours while appreciable amounts are detected only at the end of 48 hours. Thus, it appears, the reaction of induced defence becomes manifest after an induction period of 36 hours. Compared to the other known induced reactions in plants this appears to be a long induction period. In the black leg disease of potato, the tubers infected by

Bacillus atrosepticus van Hall, with the help of a needle prick develop an increased temperature within 10 hours. A similar situation is seen in the hyperthermic reaction of potato infected by *Phytophthora infestans* (Mont.) de By.

TABLE 4

Orchinol content of zones of tissue that are in immediate contact with the parasite, after different induction periods.

(After Gäumann & Hohl, 1960)

Induction period in hrs.	Orchinol content γ/g
12	0
24	0
48	28

The orchinol content increases with time, and a maximum concentration is reached eight days after the parasite has come into contact with the host tissue. Within the bulb, as seen from Table 5, segments taken from the periphery of the bulb have greater orchinol content. Thus a piece in close proximity to the fungus has an orchinol content of 920 γ per gram fresh weight while a segment away from the fungus has only 45 γ per gram fresh weight. A concentration of as high as 5×10^{-2} molar of orchinol seen in the outer tissues of the orchid bulb i e., near the focus of infection, gives a good protection against the spread of the parasite. As one goes progressively away from the periphery into the interior, there is a gradual decrease in the concentration of this substance. Nevertheless, the reaction mechanism for the synthesis of orchinol has been set in motion in all the tissues of the orchid bulb. Here we have a case of not just tissue immunity but also organ immunity. Around the focus, there is an immunization barrier to a depth of 6 mm with the highest concentration of orchinol, which is responsible for localising and containing the fungus in the periphery of the bulb. (Gäumann and Hohl, 1960).

Is orchinol an antibody?

Gäumann would like to call orchinol an induced antibody. He adduces the following reasons in support of this view. "In the sense of the classical definition of human and veterinary medi-

TABLE 5

The orchinol content in γ/g fresh weight, at different heights of a column of bulb tissue of *Orchis militaris* L. brought under the influence of *Rhizoctonia repens*, strain isolated from *Orchis militaris*. (After Gäumann & Hohl, 1960)

Induction period in days	Segments of 2 mm thick numbered from below above					
	1	2	3	4	5	6
1	0	0	0	0	0	0
2	28	10	0	0	0	0
5	200	112	15	Trace	0	0
8	920	380	160	50	35	45
12	650	540	460	110	100	80

cine, orchinol cannot be classed as an antibody; for medicine currently recognises only γ-globulins and their immediate relatives as antibodies and limits, therefore, the expression "antibody" to this particular group of substances. Orchinol chemically belongs to another group of compounds; it is much less complex and possesses less specificity. Nevertheless, we would define orchinol, by reason of its method of production and functional importance, as a genuine induced antibody. To do so, the classical medical definition of antibodies must be broadened: we drop the restriction to a specific group of substances and instead put the emphasis on the function of the newly built substance. An antibody in the biological sense would, therefore, be defined as a characteristically built substance created by the host in reply to an infection which in turn acts against the pathogen which initiated its elaboration."

On the basis of the above definition it would appear that orchinol is the first antibody from plants that has been successfully isolated in the crystalline form (Gäumann & Kern, 1959).

Antitoxic Defence Reactions

In principle, antitoxic defence reactions are meant to neutralise the toxins and necrogenous substances formed as a result of host-parasite interaction, or to localise them by histogenic demarcations (Gäumann, 1950). Both the biochemical and histogenic

protection are known to occur in man whereas plants have only the latter possibility at their disposal. Recently, however, there are one or two reports which serve as examples of biochemical antitoxic defence operating in plants. Unlike in the case of man and animals, these reactions by no means afford complete protection or impart immunity to the plant against the pathogen or its toxins. Yet, their very occurrence is sufficient to indicate the fact that, in principle, plants do possess, though in a limited way, the capacity for biochemical antitoxic defence.

Detoxication of fusaric acid

Tomato plants rapidly metabolise fusaric acid when it is administered to them. If tomato cuttings are allowed to take up a definite amount of fusaric acid whose carboxyl group has been labelled with C^{14} atom, then it is possible to account for about 85% of the original radioactivity in the extracts of these shoots made after 48 hours (Sanwal, 1956; Kluepfel, 1957).

One of the conversion products of fusaric acid detected in the tomato shoots, which varies quantitatively depending on the plant variety, is now definitely known to be a non-toxic substance. It has been identified as N-methyl fusaric acid amide ion which in the plant appears to be saturated with organic acids (Kluepfel, 1957).

N-methyl fusaric acid amide neither induces toxic symptoms on cut shoots of tomato, nor does it inhibit the spore germination of *Ustilago zeae* (Beckm.) Ung., or respiration of yeasts. The methylation of the fusaric acid molecule at the N-atom of the pyridine ring leads to a detoxication and therefore constitutes an antitoxic defence reaction.

In animal cells, many mechanisms of antitoxic reactions are known (Williams, 1949). For example, the pyridine ring is known to undergo methylation and is thereby rendered harmless. In dogs, pyridine, a highly toxic compound, when injected undergoes methylation and is excreted out of the body as a harmless substance. Again, nicotinic acid when introduced in the animal body undergoes transformation to trigonelline (N-methyl nicotinic acid). N-methylation as a detoxication mechanism has been demonstrated for the first time in plants with the toxin fusaric acid (Kluepfel, 1957).

Of the three varieties of tomato studied by Kluepfel to demonstrate this phenomenon the wilt resistant variety, Red Currant,

was seen to inactivate about 20 to 24% of the administered fusaric acid by N-methylation. The two wilt susceptible varieties, Bonny Best and Tuckswood, could methylate only about 8% of the fusaric acid (Table 6). Thus this defence character i.e., antitoxic defence reaction, finds expressions in the degree of susceptibility of tomato varieties to the toxin and thereby perhaps to the disease.

TABLE 6

The distribution of radioactivity in the different chemical fractions of extract of tomato varieties treated previously with C^{14} marked fusaric acid. (Adapted from Kluepfel, 1957)

	Tomato variety	Total recovered radioactivity in %	Loss in %	Basic fraction* in %
I.	Bonny Best ..	86·4	13·6	8·8
II.	Bonny Best ..	85·8	14·2	8·4
I.	Tuckswood ..	84·0	16·0	7·8
I.	Red Currant ..	84·5	15·5	21·5
II.	Red Currant ..	84·4	15·6	24·2
III.	Red Currant ..	79·0	21·0	30·3

* The basic fraction consists of N-methyl fusaric acid amide and gives percentage of detoxication.

Antagonism to fusaric acid

We have already seen that the toxicity of fusaric acid to certain bacteria and fungi is due to its interference with the functioning of a substance essential for their metabolism. It is of interest to know, whether a similar relationship could be shown in tomato plants, as we are primarily interested in fusaric acid as a wilt toxin. This problem presented us with a lot of experimental difficulties. Moreover, it is known that the antagonistic effect between a specific metabolite and its antimetabolite may be shown with bacterial species but not in animals or human beings, or *vice versa* (Woolley, 1952). But our experiments proved successful in a limited way.

The toxic injury of fusaric acid to cut shoots of tomato plants could be partly removed by the factor isolated from yeast extract (Kalyanasundaram, 1960). With 225 mg. of the toxin per kg. fresh weight of the tomato shoots and a particular amount of the antagonist there is a 70% reduction in the leaf injury. With a lower toxin dosage and correspondingly smaller amount of the factor

TABLE 7

Symptom production by fusaric acid on tomato in the presence of the antagonist. Concentration of the toxin in all experiments was $2 \cdot 5 \times 10^{-3}$ molar. (After Kalyanasundaram, 1960)

Exp.	pH of test solution	dose fusaric acid in mg/kg fresh wt.	Wilt Index*			
			fusaric acid		fusaric acid + antagonist	
			Stem	leaves	Stem	leaves
1.	.. 4·3	225	2·20	1·87	2·20	0·55
2.	.. 4·3	225	1·85	1·90	1·80	0·55
3.	.. 4·3	150	1·10	2·30	0·60	0·60
4.	.. 4·3	150	1·10	2·20	0·70	0·90
5.	.. 6·5	150	0·10	1·10	0·00	0·70

*The Wilt Index is a measure of the damage to tomato plants where 0 indicates no damage and 4 complete collapse of the plant.

there is nearly the same reduction in the leaf injury. However, the situation appears to be different with the stems of these plants. Only with the lower dose of the toxin there is some reduction to the injury of the stem (Table 7).

It was concluded that a part of the injury to tomato shoots caused by fusaric acid is attributable to its antimetabolite character. This is most conspicuous in the leaves, where damage caused by fusaric acid is little affected by pH changes. Moreover, the leaves, being the active centre of metabolism, offer more reactive groups than the stem. The discrepancy between stem and leaf injury is also due to the problem of non-uniform translocation of the toxin and the antitoxin in the plant.

Within the wide phanerogamic host range, the sensitivity of the various host species to fusaric acid differs both quantitatively and qualitatively (Gäumann, 1957). Table 8 gives for several host plants the quantities of fusaric acid needed to produce injuries of an intensity of 1·5 on a scale of 0 to 4, in which 0 indicates no injury and 4 indicates complete collapse of the plant. This injury threshold of 1.5 is termed *dosis minima*. Rye, maize and peas react only mildly to fusaric acid; their *dosis minima* is about 10 times that of beans, rice or tomato. Cotton plants react more sensitively, their *dosis minima* being about 1/100 that of rye, maize or peas.

TABLE 8

The sensitivity of cuttings of various host species to fusaric acid (After Gäumann, 1957)

Test plant	Dosis minima* mg/kg fresh weight
Rye, *Secale cereale* L.	1000-2000
Maize, *Zea mays* L.	1000-2000
Peas, *Pisum sativum* L.	1000-2000
Beans, *Phaseolus vulgaris* L.	100-200
Rice, *Oryza sativa* L.	100-200
Tomato shoots, *Lycopersicon esculentum* Mill.	150
Cotton, *Gossypium herbaceum* L.	10-20

*Minimum quantity that produces injuries of an intensity of 1·5 on a scale of 0 to 4, in which 0 indicates no injury and 4 indicates collapse of the plant.

On the basis of the antimetabolite nature of fusaric acid it would appear that the quantity of this toxin needed to cause this injury to any organism or organs would depend on the proportional presence of this antagonist (metabolite). This could perhaps explain the differing sensitivity of various host plants to fusaric acid mentioned above. Thus, the quantity of the antitoxin naturally present in the tissues of the plant species might determine their resistance or susceptibility to a given dose of the toxin, which plays a decisive role in pathogenesis.

Induced Tolerance

If the plant is unable to localise the pathogenic agent by the anti-infectional or antitoxic defence reactions, a new form of protection develops i.e., by a desensitization of its own body. It no longer reacts in a perceptible way to the pathogenic agent, and therefore does not become diseased.

This biological phenomenon perhaps finds expression in those instances where we hear of people developing immunity to poisons by habituation or acclimatization. In medicine, this is perhaps exemplified by typhoid carriers. In many cases of typhoid fever, the clinical cure does not mean a true microbiological healing or an elimination of the pathogen. On the contrary, the parasite remains in full vitality and infectivity for other individuals, in certain organs of the body, but the body no longer reacts to it as formerly with the typical symptoms; it has become tolerant.

The botanical examples for this type of defence reaction are afforded by the virus diseases. In plants the pathogen may occupy the whole organism completely, but nevertheless, is tolerated by it without manifest symptoms. If, for example, the virus of North American ring spot of tobacco be transmitted to fresh tobacco plants by rubbing the leaves, the typical symptoms develop on the infected leaves after three days. The virus spreads from the infected leaves into all the younger parts of the plants except the growing point. Then the ring spot symptoms gradually begin to fade, those in the newly formed leaves and lateral branches become increasingly faint, and finally, after some weeks, no definite symptoms of the disease remain. The leaves are merely somewhat darker green, thicker, and more leathery than those of virus-free plants. Meanwhile, the originally diseased leaves have fallen off but the plants have 'preserved themselves'. They are clinically cured but remain virus carriers.

There is also an indication of 'induced tolerance' developed in plants, to a specific phytotoxin. It has been demonstrated by Gäumann and Naef-Roth (1953) that tomato plants, when subjected to sub-lethal doses of lycomarasmine over a period of time, develop a resistance to this toxin. The plants are no longer as sensitive to this toxin as they would have been if they were not desensitized with sub-lethal doses of the toxin. It looks as though the plant tissues have developed a 'tolerance' to this toxin.

The limited examples I have described serve to emphasise the fact that, basically, the reactions of plant and animal tissues to pathogenic invasion are similar. Only, the practical demonstration of this becomes difficult in plants because of the peculiar organisation of the plant body.

EXPLANATION OF PLATE 1

FIG. 1. Necrosis of the vascular bundles of the leaf of Spanish chestnut (*Castanea sativa*), caused by the toxin diaporthin, a product of *Endothia parasitica*. (After Gäumann, 1954). FIG. 2. Mycelial growth of *Rhizoctonia solani*, isolated from *Solanum tuberosum*, over a segment of the bulb of *Orchis militaris*. (After Gäumann, Nuesch & Rimpau, 1960). FIG. 3. Zone of inhibition of the mycelial growth of *Rhizoctonia mucoroides*, isolated from *Vanda suavis*, by a segment of the bulb of *Orchis militaris* (After Gäumann, Nüesch & Rimpau, 1960). FIG. 4. Competitive antagonism between the factor isolated from yeast extract (vertical strip) and dehydrofusaric acid (top horizontal strip), and fusaric acid (bottom horizontal strip). Test organism is *Candida vulgaris*. (After Kalyanasundaram, 1960). FIG. 5. Principal damage to tomato leaf by lycomarasmine; note necrosis of the intercostal fields, the leaf veins being unaffected. (After Gäumann, 1951). FIG. 6. Chlorotic lesions produced in a tobacco leaf by traces of 'wildfire' toxin. (After Braun, 1955).

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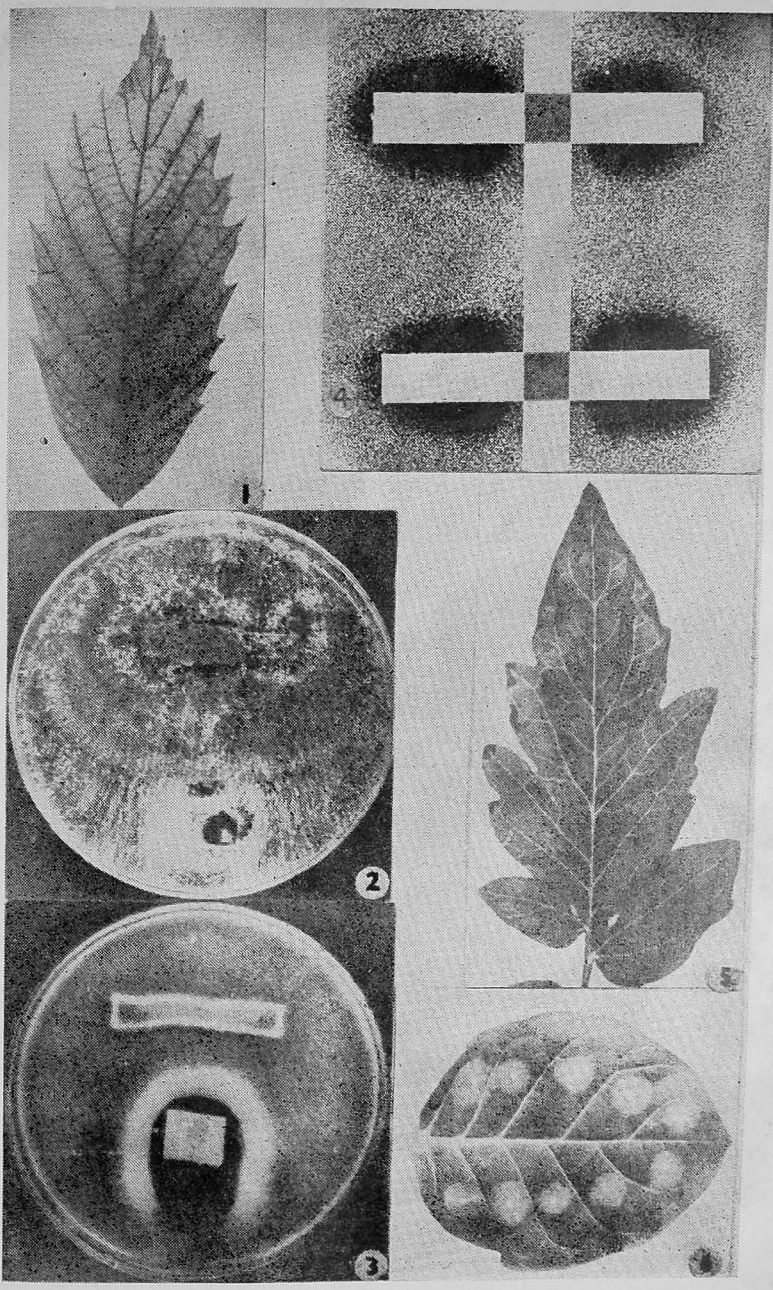

FIGS. 1-6.

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Population Dynamics of the Root-Knot Nematode *Meloidogyne ingonita* with reference to Field Conditions*

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ABSTRACT

An account of the population dynamics of the root-knot nematode, *Meloidogyne incognita* for a period of two years with reference to two fields, with different conditions is given. The density of the nematode population varied with reference to field conditions such as the fallowness of the field in dry summer, irrigational practices adopted, the presence of the susceptible host plants during the various seasons of the year, the presence of weeds which served as off-season hosts for the nematodes as well as the monsoon rains. Two indicator host plants were employed during the studies. The degree of susceptibility of the two indicator plants to the infection of the root-knot nematode varied. Continual irrigational practices throughout the year helped the presence of the nematode population all the year round. The nematode population seemed to increase from year to year.

Introduction

Godfrey (1924, 1926) in his studies reported about the depth distribution and the dynamic nature of the root-knot nematode population. The environmental relations of the root-knot nematode *Heterodera radicicola* were studied by Godfrey and Hoshino in 1933.

Franklin (1937) gave an account of the survival of the root-knot nematode *Heterodera marionii* in England. Tyler in 1938 studied the egg output of the root-knot nematode. Further information regarding the nature of the nematode populations in relation to field conditions, in the various parts of the west, was given by many workers; (Goheen and Williams, 1955; Kincaid and

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Reeves, 1943; Sasser 1954; Sasser and Nushaum, 1955; Watson and Geff, 1937; Tyler 1933; Giles and Hutton, 1958, etc.).

During the period 1955-1958 the author observed in his field surveys that the incidence of the root-knot nematode infection in the various plants varied from field to field. It was therefore decided to conduct experiments to study the root-knot nematode population with reference to field conditions.

Methods and Materials

The population studies were carried out in two fields, one at Guindy (Field No. 1) and the other at Poonamalee (Field No. 2). The field at Poonamalee was under cultivation throughout the year, with the help of monsoon rains during the rainy season and with the help of well water in summer. An electric pump-set served the purpose in summer. The field at Guindy was dependant only on the monsoon rains, being fallow in summer.

In each field an area (1/20th of an acre) was chosen to collect soil samples. Several plots of 12′ × 12′ area were demarkated and soil samples from these areas were collected in 8″ × 1″ metal tubes. The collections were made at random, covering all the directions of the field. The soil samples were then mixed well and distributed into 4″ pots with drainage holes at the bottom. Twentyfive 4″ pots were thus filled up. Surface sterilized seeds of Tomato and beans were sown in the pots and allowed to grow, with moisture at 50% of the moisture holding capacity of the soil.

Simultaneously another set of soil samples, for population studies, was collected from the same areas in 8″ × 1″ tubes. The collections were randomized. Fifty samples were thoroughly mixed and five aliquots were taken to determine the nematode population. There were five replicates in this study, each having five aliquots of soil from each field. The population studies were carried out for a period of two years (1956-1957). The data for the nematode population and for the root-knot produced in the two host plants employed are given in the tables 1 and 2 respectively.

Discussion and Comments

(i) *The nematode activity*

A perusal of the data in Table 1 shows that the nematode activity was absent in summer (April to July) in the field at

TABLE I

Seasonal data of the nematode population for two years in two fields *

Months	Field No. 1 (Guindy)				Field No. 2 (Poonamalee)			
	1956		1957		1956		1957	
	Juveniles	Egg Masses	Juveniles	Egg Masses	Juveniles	Egg Masses	Juveniles	Egg Masses
January	42	15	58	18	52	60	65	75
February	30	8	41	15	45	51	58	62
March	10	3	15	8	28	30	42	42
April	—	—	—	—	100	21	112	30
May	—	—	—	—	145	180	151	205
June	—	—	—	—	200	115	222	138
July	—	—	—	—	250	100	278	115
August	15	5	21	10	180	75	215	90
September	75	15	92	21	120	51	138	62
October	60	22	71	33	80	68	91	81
November	35	31	46	40	52	81	69	96
December	20	52	31	59	21	105	32	123

* Average amount per replicate. Each replicate consisted of 5 aliquots.

Guindy. With the advent of rains in August the nematode activity started and lasted till late in December or early in January. During the period 1956-1957 the rains started late in August and were moderate in September. During October to December they were heavy and in January the amount diminished. By February the rains stopped but the soil was wet till early March. From April to July the soil was dry.

The juvenile count shows a variation during the various seasons in 1956-1957, suggesting its dependance on the moisture in the soil.* During August, with the starting of the rains the soil moisture varied from 25% to 35%, while in September it varied from 35% to 75%. The juvenile count increased from 15 to 75 and 21 to 92 during the same months in 1956-1957 respectively. During the period from October to December there was heavy down pour due to monsoon rains, which resulted in a water-logged condition of the soil. This condition was met with a poor nematode activity as seen from the counts during that period, viz., 60 to 20 and 71 to 31 in 1956 and 1957 respectively. The reduction in the above counts may be due to the fact that water logging precluded the soil air, necessary for the nemetode activity. It may be that under these conditions, a lesser number of juveniles developed from the egg masses and migrated into the soil, or that a majority of the juveniles quickly invaded the roots, so that the few that were observed were late hatchings.

The count of the egg masses in this field increased during the rainy season (September to December) from 5 to 52 and 10 to 59 in 1956 and 1957 respectively. The count decreased during January to March from 15 to 3 and 18 to 8 during the same years. The egg masses were absent during summer i.e., from April to July. Field observations showed that the egg masses projecting out of the roots, dislodged from the roots into the soil during the rainy season, during summer the soil samples contained only crumpled portions of the egg-masses and these when soaked in water did not give rise to any juvenile, indicating that dry summer conditions killed eggs in the egg masses.

In the case of the field at Poonamalee (Field No. 2) (Table 1) the juvenile activity was found throughout the year. Fairly high

* The moisture content of the soil samples from the fields were determined in the Laboratory. Their figures, however, are not given in the table.

counts were seen from April to September, (right through the summer) and the number decreased during the rainy season, October to December. The egg masses were also present through the year Table I shows that during summer (May to July) high counts with reference to egg masses were obtained, the counts decreased from August to September and increased from October to December. Again there was a reduction in the counts of the egg masses from January to April. The moisture in this field did not go below 35% even in summer, when the water supply was maintained by means of an electric pumpset. It was observed that summer was the peak period of nematode activity in this field. With the starting of the monsoon rains the activity slowly diminished.

In this field vegetables were grown in summer and the plants were uprooted in May, after which the land was prepared for the next crop. The plants were not completely removed from field but were ploughed in to serve as manure. This resulted in the egg masses being retained in the field. The egg masses gave rise to juveniles, when the field was watered. The egg masses were in larger numbers when the land was prepared for the next crop. During August and September the field was full of seedlings. It was quite likely that the presence of the seedlings which released their root exudates, probably activated the egg masses to develop into juveniles. (Gadd and Loos 1941; Lindford 1939; Weiser, 1955) Hence a reduction in the egg masses was observed with a corresponding increase in the juveniles.

The behaviour of the nematode population in this field, during the rainy season (October to December) was similar to that in the Field No. 1, since both the fields received water from the monsoon rains.

(ii) *The Root-knots*:

The data given in Table II shows the following:—

The root-knot counts varied during the various seasons of the year in the two indicator host plants viz., Beans and Tomato. Since the root-knot nematode *Meloidogyne incognitea* is responsible for the root-knot infection, the amount of the root-knots produced naturally depends upon the number of the nematodes present in the soil. As already stated (cf. methods and materials) the indicator plants were maintained in the same soil collected from the same places from the two fields. A comparison of the

TABLE II

Average root-knot counts in two host plants subjected to the infection from the soils of two fields.

Months	Beans				Tomato			
	Field No. 1 (Guindy)		Field No. 2 (Poonamalee)		Field No. 1 (Guindy)		Field No. 2 (Poonamalee)	
	1956	1957	1956	1957	1956	1957	1956	1957
January	65	78	78	98	90	98	120	148
February	42	50	70	82	52	65	108	121
March	20	29	62	75	31	42	88	99
April	—	—	45	56	—	—	75	82
May	—	—	268	302	—	—	356	408
June	—	—	205	264	—	—	289	326
July	—	—	135	202	—	—	204	264
August	35	42	98	138	60	72	146	198
September	58	74	75	106	81	98	108	127
October	75	98	94	128	100	120	156	202
November	98	125	149	198	126	148	215	265
December	120	154	228	295	148	162	306	362

tables I and II shows that the root-knot incidence varies, depending on the density of the nematode population in the soil. The absence of the root-knots in summer in the case of the soil from Guindy (Field No. 1) was therefore due to the absence of the Nematodes (Table I; Field No. 1). On the other hand cultivation in summer resulted in the occurence of the nematode with a corresponding root-knot incidence as seen in the case of the soil from the field at Poonamallee (Table II, Field No. 2).

The two fields (Field No. 1 at Guindy and Field No. 2 at Poonamalee) were constrasting, in that a break occured in summer in Field No. 1 (at Guindy) due to the fact that the land was dry with neither the nematodes nor the plants, whereas in the Field No. 2 (at Poonamallee) there was no such break, as it was well-watered so that throughout the year, both the host plants and the nematodes existed.

The incidence of the root-knots in August in Field No. 1 (at Guindy) after a break in summer can be explained as follows:

During summer, the field was full of weeds mostly with *Acalypha indica*, etc., susceptible to the root-knot nematode attack. The root-knots of these weeds were very thick, protecting the nematodes within them from dry summer conditions. Steckhoven (1941) while quoting Bessey (1911) pointed out that the extreme conditions were tided over within the root-knots, once the infection had taken place, since the roots never attained the same temperature as the soil.

The first ploughing in summer did not completely cut the roots of these weeds, so that when the land was ploughed again during August-September rains, the egg masses developed into the infective juvenilles which carried the infection to the crops of the next season. Here is a case where the weeds acted as the off-season hosts for the root-knot nematode to tide over the unfavourable summer conditions, with the result, that, the summer was the period of rest for the nematodes for want of moisture in the soil and the natural hosts for infection.

The two indicator host plants (Beans and Tomato) varied in their root-knot counts, being larger for tomato than for beans, thereby showing that the former was more susceptible to the infection under identical conditions.

Conclusions:

The nematode population varied according to the environmental conditions, depending upon factors like moisture and the presence of the host plants. The more extreme the soil conditions, the greater was the variation in the population. When the soil conditions did not vary much the population did not fluctuate drastically.

Irrigational practices influenced the nematode population during the different seasons. Cultivation all through the year with water facilities in summer prevented break in the population during any time in the year (Field No. 2), whereas, dry summer with no cultivation led to the absence of the nematode population (Field No. 1).

Under the influence of natural conditions (Field No. 1) weeds served as a sort of reservoir hosts, and helped the nematode to tide over the summer draught. Weeds helped to carry over the infection to the next round of crops. Under natural conditions August to September were the periods of high juvenile activity, while in November and December the egg masses were found in greater numbers in the soil. Summer with fallow conditions was a period of rest. (cf. Field No. 1; Table I).

When cultivation was carried out throughout the year (Field No. 2), a high juvenile activity occurred in summer (Table I), while the egg masses were present throughout the year.

Flooded condition of the soil (in rainy season) was not condusive to high juvenile activity; nor the extreme dryness and heat in summer. Extreme dryness and heat of the soil in summer killed the nematodes within the root-knots, when the roots were cut by ploughing. On the other hand when the whole plant (weeds, etc.) were only uprooted during ploughing (the roots not being cut) the nematodes managed to come over the unfavourable dry summer conditions within the root-knots, since, the whole plant though, uprooted was still in the field and continued to live. The practice of leaving the uprooted plants in the field, as green manure prevented the complete elimination of the nematode population from the fields. Under such conditions, the best possible method of checking the onset of fresh infection of the next round of crops will be, the collection of all the weeds (serving as off season hosts in summer) from the fields and burning them completely to kill the host with the parasite.

The population density directly reflected the amount of production of root-knots in the susceptible plants. Among the susceptible hosts, tomato suffered more than beans. The nematode population increased from year to year producing larger and larger number of root knots as shown by the counts in the two susceptible host plants.

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An Amperometric Method for the Estimation of Copper

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ABSTRACT

An accurate method of estimation of copper by the amperometric technique has been developed. The method is based on the precipitation of copper with resacetophenone in a 0·5 M sodium acetate—0·1 M potassium nitrate supporting electrolyte. The titration is performed at—0·8 V vs. S.C.E. Cations like Zn, Ni, Cd, Mg and Pb do not interfere.

Introduction

For the estimation of copper a large number of amperometric titration methods involving organic precipitating agents have been reported in the literature (Kolthoff and Lingane, 1952, p. 923 ff). Among them oxine, quinaldic acid, quinoline 8 carboxylic acid, cupferron and salicyl-aldoxime may be mentioned. The aim of the present investigation is to examine the possibility of using resacetophenone for the amperometric estimation of copper. As the reagent is one that can be easily prepared from readily available laboratory chemicals and is suitable for copper estimation without interference from many bivalent metals, the method commends itself as worthy of investigation.

Chemistry of the method

Resacetophenone 2, 4-$(OH)_2$ $C_6H_3.CO.CH_3$ can be easily prepared by the action of glacial acetic acid on resorcinol. (Cooper, 1955), and can be purified by recrystallisation thrice from boiling distilled water containing some hydrochloric acid when it appears as faint brown plates melting at 143-144°. Two

moles of the reagent complex with one mole of copper (Ramanujam, 1953), the formula of the complex being $(C_8H_7O_3)_2$ Cu. Previously resacetophenone has been used by Neelakantam and Row (1942) for detecting boron, and by Cooper (1937) for detecting iron. Ramanujam (1953 and 1956) has used it for the gravimetric and volumetric estimation of copper in presence of Zn, Ni, Co, Mg, Pb, Cd and Mn in acetate buffered solutions. The polarographic reduction of resacetophenone in alcoholic ammonium chloride solutions has been studied by Valyashko and Rozun (1948).

Selection of the supporting electrolyte

Ramanujam (1955 Thesis), during his studies on gravimetric estimation of copper, has concluded that an ammoniacal medium is not suitable because of the slight solubility of the precipitate in that medium. He found that an acetate buffered solution is preferable and recommends a pH range of 5·6 to 6·2. The precipitate is soluble in alkaline and acetic acid solutions. Hence pure sodium acetate solution is taken for the polarographic study of copper and resacetophenone. The supporting electrolyte also contains some potassium nitrate.

Preliminary Polarographic study

A Tinsley Model recording polarograph has been used in this investigation. The capillary used has an $m^{2/3}\ t^{1/6}$ value of 1·489 $mg^{2/3}\ sec^{-1/2}$. Polarography of copper has been studied for various concentrations in 1 M and 0 5 M sodium acetate solutions containing 0·1 M KNO_3. 0 01% gelatin solution is used for suppressing the maxima (Kolthoff and Lingane, 1952, p. 494). Table I gives the values of diffusion currents, halfwave potentials and diffusion current constants for various concentrations of copper. Figure 1 shows the strict linearity of the diffusion current with copper concentration. A polarogram of copper in 0·5 M sodium acetate containing 0·1 M KNO_3 and 0·01% gelatin is reproduced in Figure 5. The diffusion current plateau above —0·5 V vs. S.C.E. is well defined. 0·5 M sodium acetate base electrolyte is preferable to 1 M sodium acetate solution as it gives more than 2·5 times the diffusion current for the same concentration of copper. Concentrations of sodium acetate smaller than 0·5 M did not improve the diffusion current values further. Hence a base electrolyte which is 0·5 M in sodium acetate and 0·1 M in potassium nitrate is chosen for the amperometric study.

TABLE I

Polarography of Copper

Supporting Electrolyte: 0·5 M sodium acetate + 0·1 M KNO_3
$m = 1{\cdot}187$ mg/sec. $t = 5{\cdot}5$ sec.

$m^{2/3} = 1{\cdot}121$ $t^{1/6} = 1{\cdot}328$

$m^{2/3}t^{1/6} = 1{\cdot}121 \times 1{\cdot}328 = 1{\cdot}489$ $mg^{2/3}$ $sec.^{-1/2}$

No.	Concentration of copper in millimoles C	Diffusion current (corrected) id microamperes	Half-wave potential $E_{1/2}$ -V vs. S.C.E.	$\frac{id}{C}$	Diffusion current constant I
1.	1·00	3·5	—0·110	3·500	2·359
2.	2·00	7·2	—0·112	3·600	2·421
3.	2·85	10·4	—0·116	3·640	2·460
4.	3·80	14·2	—0·114	3 737	2·519
5.	4·65	17·5	—0·115	3·762	2·536
6.	5·45	20·5	—0·116	3·761	2·536
7.	6·25	23·5	—0·118	3 760	2 534
			av —0·114	av 3·680	av 2 4807

Resacetophenone is reduced at the dropping mercury electrode with an $E_{1/2}$ of —1·49 V vs. S.C.E. in alcoholic ammonium chloride medium. [Valyashko and Rozun (1948)]. In 0 5 M sodium acetate medium this $E_{1/2}$ value is shifted to —1·8 V vs. S.C.E.

As the copper wave is well defined after —0·5 V vs. S.C.E. the constant potential for the amperometric study was fixed at —0·8 V vs. S.C.E. At this potential lead and cadmium give diffusion currents while nickel, magnesium and zinc do not. The lead wave starts at approximately —0·5 V and the diffusion current plateau becomes flat at —0·8 V vs. S.C.E. Therefore the titration curve for copper alone and in presence of Ni, Mg, and Zn will be a straight line cutting the volume axis at the end-point. In presence of Pb and Cd there will be two straight lines cutting at the end point.

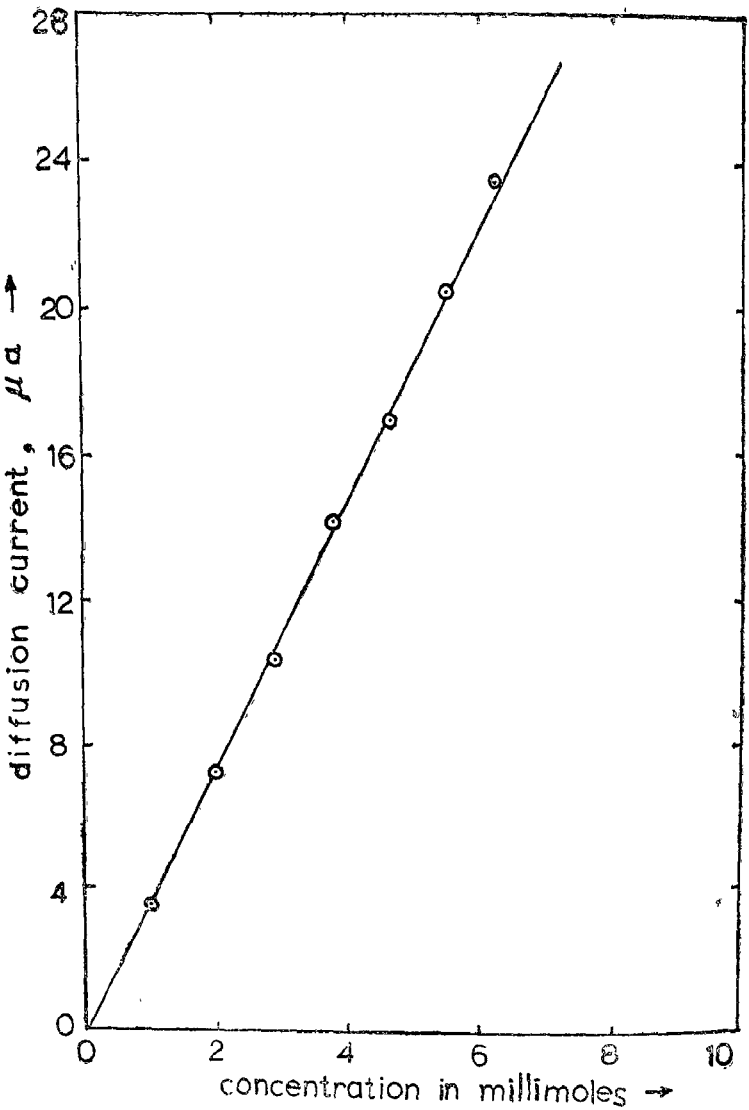

FIG. 1. id/c curve

Variation of diffusion current (id) with concentration of copper

Amperometric study

Reagents: All chemicals used are of analytical grade.

Supporting electrolyte solution: 68 g. of $CH_3.COONa.3H_2O$ and 10·1 g of KNO_3 dissolved in 1 litre of water.

Resacetophenone reagent: (0·1 M): 1·52 g of resacetophenone is accurately weighed and dissolved in 15 ml. of 95% alcohol and diluted with water to 100 ml. Standardised according to the method of Ramanujam (1955).

Metal salt solutions:

1. *Copper*: $CuSO_4 \cdot 5H_2O$: 0·05 M : 1·2489 g in 100 ml. of water. Standardised by precipitating as cuprous thiocyanate and then titrating with standard KIO_3 in presence of strong hydrochloric acid and chloroform indicator. [Vogel (1951) p. 362-3].

2. *Cadmium*: $3CdSO_4\ 8H_2O$: 0·5 g in 100 ml. Standardised by volumetric estimation after precipitation with oxine. [Vogel (1951), page 373].

3. *Zinc*: $ZnSO_4 \cdot 7H_2O$: 1 g in 100 ml. Standardised by volumetric estimation after precipitation with oxine in acetic acid solution buffered with acetate. [Vogel, (1951), p. 374].

4. *Nickel*: $NiSO_4\ 6H_2O$: 1 g in 100 ml. Standardised by gravimetric estimation with dimethyl glyoxime [Vogel (1951), p. 417].

5. *Magnesium*: $MgSO_4 \cdot 7H_2O$: 0·3 g in 100 ml. Standardised by gravimetric estimation with oxine. [Vogel (1951), p. 481].

6. *Lead*: $(CH_3COO)_2\ Pb\ 3H_2O$: 0·5 g in 100 ml. Standardised by gravimetric estimation as chromate [Vogel (1951), p. 421].

Experimental

Apparatus: A Tinsley Model recording polarograph is used. The titration vessel is a 100 ml. tall spoutless beaker and the assembly is constructed according to the instructions of Stock (1947). An external S.C.E. is used by connecting the cell with a potassium nitrate bridge. Nitrogen gas after purification (Milner, 1957) is used for de-aeration and for stirring the solution after the addition of each increment of the reagent. During the titration the gas is passed over the surface of the liquid.

Procedure: Cu^{++} solution is pipetted in to the cell and the volume is made up to 50 ml. with the base electrolyte. The solution is de-aerated for three minutes and the diffusion current is

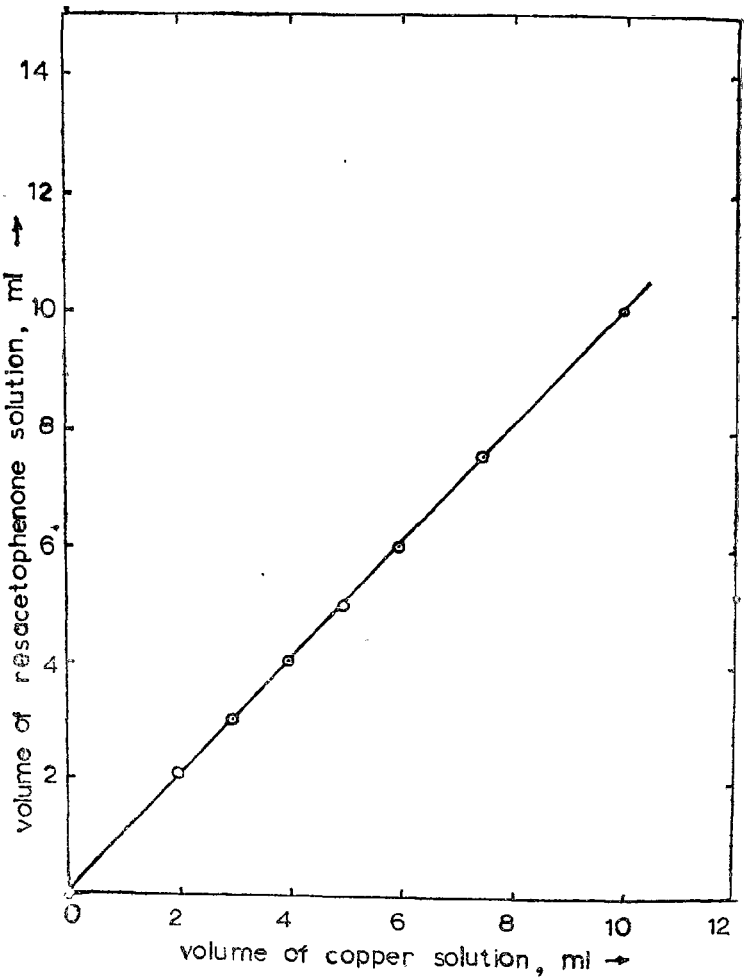

FIG. 2. Calibration curve

Volume of reagent solution required by amperometric method for different volumes of copper solution.

recorded after setting the voltage at $-0{\cdot}8$ V vs. S.C.E. The reagent is added in 0·5 ml increments with a microburette and the

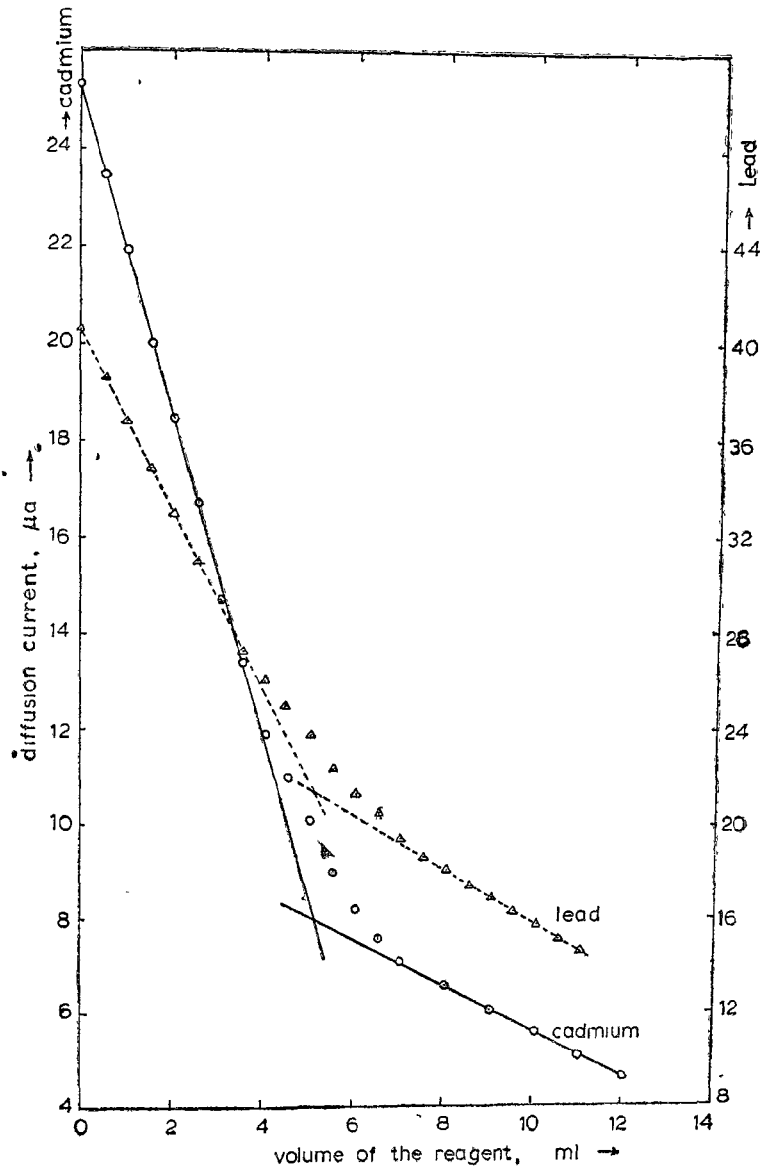

FIG. 3. Titration curves

Amperometric titration of 5 ml of copper in presence of (1) Lead (2) Cadmium. (Table III)

solution stirred with nitrogen for one minute. The precipitate is allowed to settle and after two minutes the diffusion current is recorded. The titration curve is constructed by plotting the diffusion current values against the volume of the reagent added, after correcting the diffusion currents for volume change.

Maxima suppressor gelatin is not added in the actual titration as it distorted the shape of the titration curve (Fig. 4) initially by suppressing the diffusion current. The potential is set at −0·8 V vs. S.C.E., the electrodes introduced and the titration started, so that the maxima appearing between −0·2 and −0·4 V is skipped.

TABLE II

Titration of Copper

No.	ml. of copper present	ml. of reagent required	ml. of reagent (theoretical)	Deviation ml.
1.	2·0	2·12	2·02	+0·10
2.	3·0	3·05	3·03	+0·02
3.	4·0	4·07	4·04	+0·03
4.	5·0	5·01	5·05	−0·04
5.	6·0	6·10	6·06	+0·04
6.	8·0	8·12	8·08	+0·04
7.	10·0	10·15	10·10	+0·05

Discussion

From the data presented in Table I and from Fig. 1 the strict linearity of the diffusion current with concentration of copper becomes evident. Data presented in Table II show the various volumes of the reagent consumed for different concentrations of copper and the calibration curve (Fig. 2) shows the reproducibility of the method. Data presented in Table III indicate that copper can be successfully titrated in presence of varying amounts of Zn, Cd, Pb and Mg without interference from these metals. Figure 3, represents the titration curves for 5 ml. of copper in presence of lead and cadmium. Figure 4 represents the titration curves for 2 ml and 5 ml of copper when present alone.

TABLE III

Concentration of copper in millimoles	Concentration of nickel in millimoles	ml. of reagent (Theoretical)	ml. of reagent (found)	Deviation ml.
4·0	1·72	4·04	4·06	+ 0·02
5·0	1·72	5·05	5·10	+ 0·05
4·0	3·44	4·04	4·07	+ 0·03
5·0	6·88	5·05	5·09	+ 0·04
5·0	17·20	5·05	5·11	+ 0·06
Concentration of copper in millimoles	**Concentration of zinc in millimoles**	**ml. of reagent (Theoretical)**	**ml. of reagent (found)**	**Deviation ml.**
4·0	3·48	4·04	4·07	+ 0·03
5·0	3·48	5·05	5·11	+ 0·06
4·0	6·96	4·04	4·08	+ 0·04
5·0	13·92	5·05	5·10	+ 0·05
5·0	17·40	5·05	5·12	+ 0·07
Concentration of copper in millimoles	**Concentration of magnesium in millimoles**	**ml. of reagent (Theoretical)**	**ml. of reagent (found)**	**Deviation ml.**
4·0	1·215	4·04	4·07	+ 0·03
5·0	1·215	5·05	5·06	+ 0·01
4·0	3·645	4·04	4·06	+ 0·02
5·0	6·075	5·05	5·09	+ 0·04
5·0	12·150	5·05	5·08	+ 0·03
Concentration of copper in millimoles	**Concentration of cadmium millimoles**	**ml. of reagent (Theoretical)**	**ml. of reagent (found)**	**Deviation ml.**
4·0	1·948	4·04	4·10	+ 0·06
5·0	1·948	5·05	5·10	+ 0·05
4·0	3·896	4·04	4·08	+ 0·04
5·0	7·792	5·05	5·09	+ 0·04
Concentration of copper in millimoles	**Concentration of lead in millimoles**	**ml. of reagent (Theoretical)**	**ml. of reagent (found)**	**Deviation ml.**
4·0	1·32	4·04	4·08	+ 0·04
5·0	1·32	5·05	5·09	+ 0·04
4·0	2·64	4·04	4·06	+ 0·02
5·0	2·64	5·05	5·08	+ 0·03

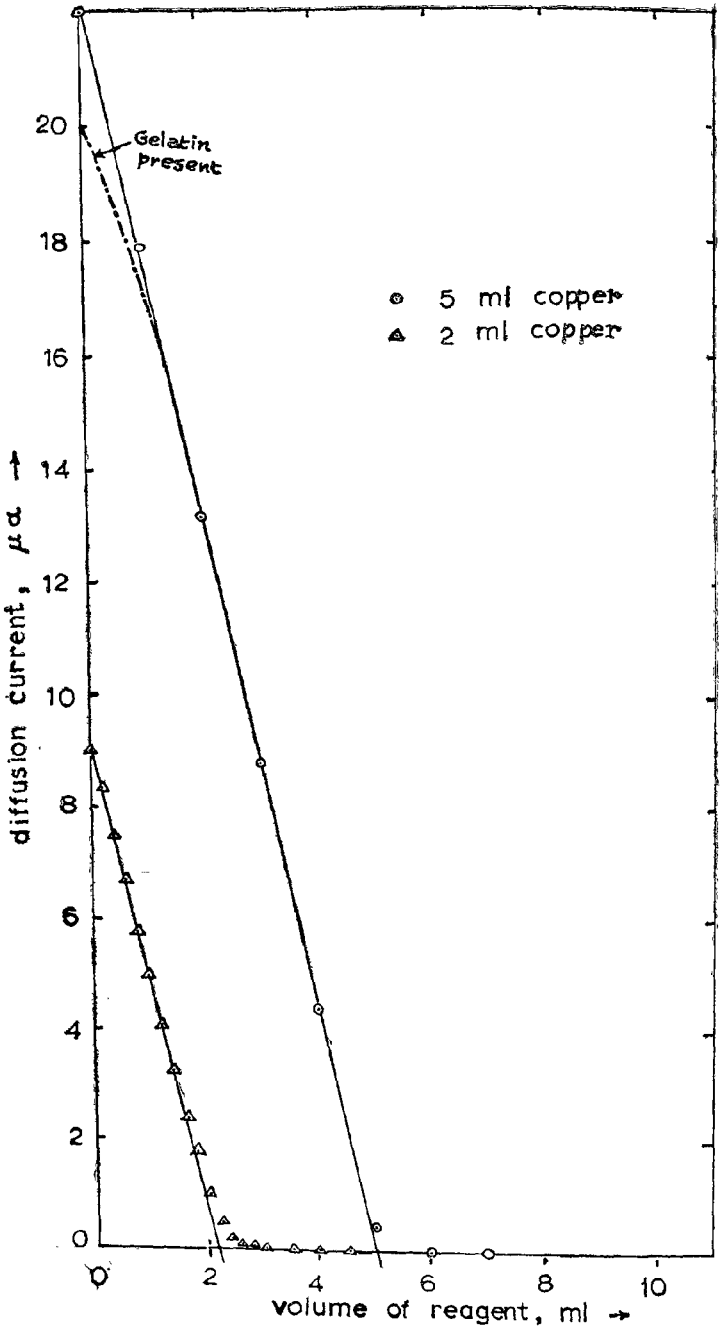

FIG. 4. Titration curves 0·5 M sodium acetate buffer
Amperometric titration curves:
1. Solution contains 2 ml. of Cu. 2. Solution contains 5 ml. of Cu.

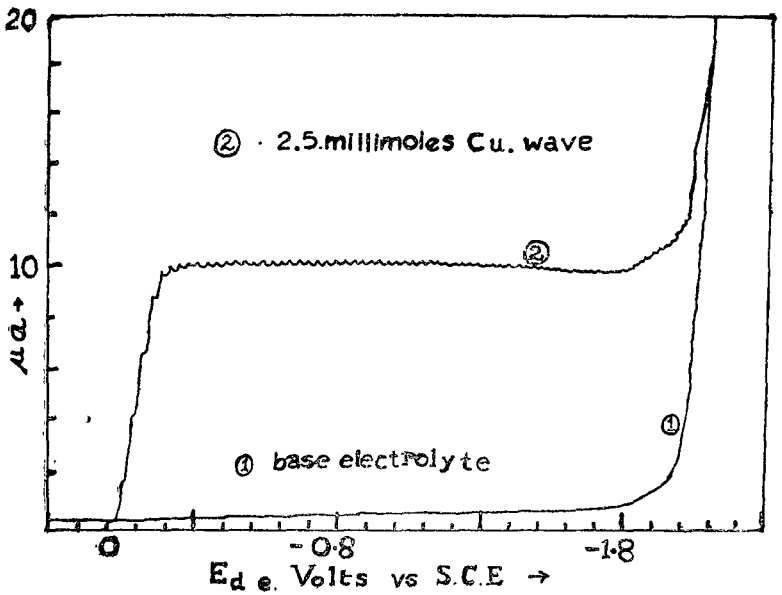

FIG. 5. Polarogram

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Studies on Root-rot Diseases of Egg-plant, Chilli and Tomato in the Madras State

I CAUSAL ORGANISMS AND PATHOGENICITY*

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Introduction :

In the Madras State the three Solanaceous crops viz., egg-plant, chilli and tomato are cultivated in different parts of the State under divergent conditions of soil, climate and environmental factors. These crops are frequently subject to wilt and root rot diseases due to soil borne fungi. Diseased specimens of these crops received from different parts of the State have most frequently yielded species of *Fusarium* besides a few other fungi. But no detailed work beyond mere isolation of the causal organisms has been done so far. The correct identity of the species of *Fusarium* associated with the diseases has not yet been conclusively established. It was also not known whether the causal organisms are confined to only one host or could infect other Solanaceous hosts. Since information on these aspects is essential in order to devise suitable remedial measures detailed investigations were undertaken.

The prevalence of wilt disease on egg-plant due to *Fusarium* spp. was first reported in the Madras State by Subramanian (1951). Damodaran (1954) reported the prevalence of egg-plant wilt in different parts of the Madras State and the causal organism was identified as *Fusarium oxysporum*. Outside Madras State egg-plant wilt was reported from West Bengal by Chattopadhyaya and Sengupta (1956). McRae (1932) was the first to report the prevalence of a wilt disease due to *Fusarium* spp. on chilli in India. Thomas (1938) reported the occurrence of wilt disease on chilli

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due to a strain of *Fusarium vasinfectum* from the Madras State. Subsequently the prevalence of chilli wilt in different districts of the Madras State has also been reported (Anon., 1954). On tomato the wilt disease occurring in South India was studied in some detail by Varma (1954) and the causal organism inciting this disease was found to be *Fusarium solani.*

It would thus be seen that information on several aspects of these root rots is either meagre or not available at all. With a view to gathering information on these diseases detailed investigations were, therefore, undertaken on the following aspects:

Survey of the prevalence and distribution of root rot diseases on these three crops in different districts of the Madras State.

Isolation of the causal organisms and identification of the isolates of *Fusarium.*

Pathogenicity tests of the various isolates of *Fusarium.*

Cross-inoculation studies.

Morphological and cultural studies.

The investigations were carried out since 1956 and the results obtained are presented in this paper.

Materials and Methods

Isolation and maintenance of cultures: The various isolates of *Fusarium* spp. used in this investigation were obtained from the diseased roots of egg-plant, chilli and tomato collected from different parts of State. In each place the diseased specimens were collected from a numbr of fields.

For the isolation of the causal organisms the diseased specimens were surface sterilized with mercuric chloride (1 : 1000) and washed in several changes of sterilized water. The surface sterilized pieces were transferred to oat agar medium under aseptic conditions. The Petri plates were incubated at laboratory temperature (26°-29°C). Fungal growth was evident around the incubated pieces within about 3-4 days. A conidial suspension of the fungus in sterile water was prepared by transferring a small portion of the growing mycelium from the colonies. The conidial suspension thus obtained was used for making single spore isolation of the cultures. After isolation the cultures were maintained in oat agar slants incubated in diffused light at laboratory temperature. The cultures were transferred to fresh agar slants once in two

months. The different isolates were identified following the key proposed by Wollenweber and Reinking (1935).

Pathogenicity studies:

(a) *Pathogen*: All the isolates obtained from three hosts viz., thirty isolates from from egg-plant, thirty five isolates from chilli and six isolates from tomato were tested for their pathogenicity on their respective hosts.

(b) *Hosts*: The seedlings of different hosts were raised in seedling pans using red loamy gardenland soil mixed with liberal doses of farmyard manure. One month old uniformly grown seedlings were transplanted in porcelain glazed pots containing inoculum.

(c) *Inoculum*: The inoculum was multiplied in sand-maize medium autoclaved at 20 lb. pressure for 2 hr. The sterilized medium was inoculated by transferring equal quantities of inoculum of the various isolates growing on oat agar slants. The inoculated bottles were incubated at laboratory temperature for a period of three weeks. At the end of the incubation period the inoculum was transferred to glazed porcelain pots containing sterilized sand and then thoroughly incorporated. An inoculum dose of 1 : 10 (inoculum : sand) was used throughout and the suitability of this dosage in obtaining successful artificial infection was ensured beforehand.

The plants were regularly irrigated with Hoagland's nutrient solution. The pots were kept in the open under pot culture conditions and the temperature ranged between 26°-29°C during the conduct of the experiment. The plants were under constant observations for mortality and other disease symptoms for a period of 10 weeks. The causal Fusaria were reisolated from the wilted plants to ensure proof of pathogenicity. The mortality per cent under each isolate was used as the criterion for assessing the pathogenicity of the various isolates.

Cross inoculation studies: The cross inoculation studies were made with 10 isolates from each of egg-plant and chilli which exhibited fairly high degree of virulence in the pathogenicity tests. All the six isolates from tomato were used in the studies.

In both pathogenicity and cross inoculation experiments two replicates with 5 plants in each replication were maintained. An

equal number of uninoculated plants was also kept as control in each experiment.

Morphological and cultural studies: Single spore cultures were used in these studies. The isolates were studied for these characters following the methods recommended by Wollenweber *et al.* (1925). The morphological characters like the shape, size and septation of different types of spores viz., microconidia, macroconidia and chlamydospores were recorded. Camera Lucida drawings of the different types of spores of the various isolates were made under the oil immersion objective. The isolates were grown in oat agar medium and cultural characters like the amount, nature and colour of the aerial mycelium, production of sporodochia, pionnotes etc., were recorded. The colour production was observed by growing the different isolates in steamed rice. The records of colour were made using Maerz and Paul's Dictionary of Colour.

Experimental Results

Survey and distribution of the disease in the Madras State:

An intensive survey was undertaken in different districts of the Madras State to ascertain the prevalence and extent of root rot diseases due to *Fusarium* spp. on the three Solanaceous hosts viz., egg-plant, chilli and tomato. The survey was conducted in the transplanted fields only. In each district a number of places were visited and in each place diseased plants from different fields were collected. The diseased specimens were brought to the laboratory for the isolation of causal organisms.

It was observed that the disease was prevalent in all places wherever these crops are cultivated. The incidence of the disease was found to range from 1-40 per cent in different regions of the State. Although the disease was observed at any stage of the growth young crops between 1-3 months old appeared to be more susceptible than the mature crops. The diseased plants exhibited various symptoms consisting of, for the most part, wilting, yellowing of the leaves and stunting of the plants. Such affected plants when pulled out and examined exhibited rotting of the root system in varying degrees. In some cases the collar region and the base of the stem were found to be shrunk. It was clear from the symptoms observed that the plants were suffering from 'root rot' rather than vascular 'wilt.' All the symptoms described above were found to be common in all the three crops viz., egg-plant, chilli and tomato.

The details regarding the places of collection, percentage of *Fusarium spp.* and other fungi isolated from the specimens collected in different regions of the Madras State are furnished in Table 1.

TABLE 1

Sources of isolates of Fusarium spp. from egg-plant chilli and tomato

Place of Collection (1)	% *Fusarium* spp. (2)	% Other fungi (3)
EGG-PLANT		
Nilgiris district:		
Pomological Station, Conoor	95·0	5·0
Coimbatore district:		
Vellalapalayam	93·3	6·7
Tudialur	95·0	5·0
Lakkampatti	95·0	5·0
Salem district:		
Velur	91·7	8·3
Pothanur	95·0	5·0
North Arcot district:		
Gudiatham	91·7	8·3
Kannadikuppam	92·5	7·5
Lakshmipuram	92·5	7·5
Madurai district:		
Palani	92·5	7·5
Kalayamuthur	98·4	1·6
CHILLI		
Coimbatore district:		
Kuppakonampudur	92·3	7·7
Velandipalayam	96·0	4·0
Satyamangalam	97·5	2·5
Alukuli	97·5	2·5
Vellalapalayam	100·0	0·0
Lakkampatti	95·0	5·0
Tirunelveli district:		
Kovilpatti	97·0	3 0
Talayuthu	95·0	5 0

Place of Collection (1)	% *Fusarium* spp. (2)	% Other fungi (3)
Ramanathapuram district:		
Meenampatti	97·0	3·0
Sattur	100·0	0·0
Madurai district:		
Uthamapalayam	97·5	2·5
Cumbum	95·0	5·0
Chingleput district:		
Saidapet	95·0	5·0
TOMATO		
Coimbatore district:		
Veerakeralam	90·0	10·0
Central Farm, Coimbatore	95·0	5·0
North Arcot district:		
Jalarpet	90·0	10·0
Vellore	90·0	10·0
Madurai district:		
Dindigul	90·0	10·0

Other fungi: Species of *Pythium, Aspergillus, Rhizopus, Rhizoctonia* and *Sclerotium rolfsii.*

It will be seen from the results above that more than 90 per cent of the isolations are species of *Fusarium* in the case of all the three crops. It is, therefore, evident that the genus *Fusarium* appeared to be important in the causation of root rot diseases on these three important Solanaceous crops. The results further revealed the ubiquitous distribution of the genus *Fusarium* in the cultivated soils of different districts of the Madras State.

After a careful study of morphological and cultural characters of the various isolates in detail they were identified following the key of Wollenweber and Reinking (1935). It was observed that the characters of all the isolates were found to conform closely to the characters of the Section *Martiella*. Within this Section all the isolates have been identified as belonging to two varieties and one species of *Fusarium* viz., *F. solani* v. *minus*, *F. solani* v. *martii* and *F. solani.*

The identity of the various isolates of *Fusarium* from different hosts into *F. solani* and its varieties is given in Table 2.

TABLE 2

Identity of various isolates of Fusarium from egg-plant, chilli and tomato

Name of var. and sp. of *Fusarium*	Isolate numbers of		
	Egg-plant	Chilli	Tomato
F. solani	1, 3, 5, 6, 7, 8, 10, 11, 12, 15, 16, 17, 18, 23, 24, 25, 27, 28 & 29,	1, 8, 10, 11, 12, 15 ,17, 19, 22, 23, 25, 26, 31. 32, 33, 34,	nil
F. solani v. *minus*	2, 4, 9, 13, 19, 20, 21, 22, 26, 30,	2, 3, 4, 5, 6, 7, 9, 13, 16, 20, 24, 27, 28, 30 & 35,	1, 2, 3, 4, 5 & 6
F. solani v. *martii*	14	14, 18, 21, 29	nil
Total	30	35	6

It will be seen from the results above that *F. solani* and *F. solani* v. *minus* are predominant in the case of both egg-plant and chilli while in the case of tomato only *F. solani* v. *minus* appeared to be important. It may, therefore, be concluded that *F. solani* and its variety *F. solani* v. *minus* are primarily responsible for the the causation of root rot diseases on these crops in the Madras State.

The distribution of various members of the Section *Martiella* in different districts of the Madras State is given in Table 3.

TABLE 3

Distribution of various members of the Section Martiella in different districts of the Madras State

Name of district	Number of isolates of		
	F. solani	*F. solani* v. *minus*	*F solani* v. *martii*
EGG-PLANT			
Nilgiris ..	2	1	—
Coimbatore ..	4	3	—
Salem ..	4	1	1
North Arcot ..	3	4	—
Madurai ..	6	1	—
	19	10	—
CHILLI			
Coimbatore ..	6	8	1
Chingleput ..	2	—	—
Madurai ..	2	3	1
Ramnad ..	4	1	1
Tirunelveli ..	2	3	1
	16	15	4
TOMATO			
Coimbatore ..	—	2	—
North Arcot ..	—	3	—
Madurai ..	—	1	—
	—	6	—

Pathogenicity studies:

The pathogenic potentialities of the various isolates obtained from egg-plant, chilli and tomato were tested under pot culture conditions as per the method already described. The pathogenicity was assessed on the basis of mortality percentage of plants under each isolate. In many instances the plants were not killed outright but the isolates caused stunting effects. Plants exhibiting stunting symptoms have readily yielded *Fusarium* spp. on isolation and as such the isolates were considered as pathogenic ones. In such cases the root weights of uninoculated healthy controls were compared with root weights of diseased plants and taking the root weight of control (uninoculated) as 1 (one), the corresponding values for the root weights of diseased plants for each isolate was worked out and the values were given as 'root

rot index.' The root rot index will give a measure of loss of roots due to infection by the pathogen.

The range of pathogenicity of 30 isolates of *Fusarium* spp. from egg-plant, 35 isolates from chilli and 6 isolates from tomato was assessed and the results are set out in Table 4.

TABLE 4

Pathogenicity of various isolates of Fusarium spp. on egg-plant, chilli and tomato

Isolate numbers (1)	Var. and sp. of *Fusarium* (2)	Mean mortality per cent (3)
EGG-PLANT (30 Nos.)		
5	*F. solani*	0·0
3, 7, 8, 10, 11, 12, 16, 17, 23, 25, 28, 29	*F. solani*	25·0
1, 15, 18, 24	,,	37·5
6 & 27	,,	50·0
2, 9, 19, 30	*F. solani* v. *minus*	25·0
4, 13, & 20	,,	37·5
22	,,	62·5
21 & 26	,,	75·0
14	*F. solani* v. *martii*	25·0
CHILLI (35 Nos.)		
1, 8	*F. solani*	0·0
31	,,	20·0
15, 17 & 33	,,	40·0
11	,,	50·0
10, 19, 22, 25 & 26	*F. solani*	60·0
23	,,	80·0
12, 32 & 34	,,	100·0
35	*F. solani* v. *minus*	0·0
2	,,	20·0
3, 5, 13 & 24	,,	40·0
4, 9, 28 & 30	,,	60·0
6 & 16	,,	80·0
7, 20 & 27	,,	100·0
14 & 29	*F. solani* v. *martii*	20·0
21	,,	60·0
18	,,	80·0
TOMATO (6 Nos.)		
2	*F. solani* v. *minus*	0·0
3, 5 & 6	,,	10·0
1 & 4	,,	20·0

TABLE 5

Comparison of mortality per cent groups and mean root rot index of 30 isolates of Fusarium from egg-plant

No.	Var and sp. of *Fusarium*	Range of mortality per cent	Mean root rot index
1.	*F. solani* ..	25·0-37·5	0·44
2.	„ ..	50·0-75·0	0·36
3.	*F. solani* v. *minus* ..	25·0-37·5	0·39
4.	„ ..	50·0-75·0	0·14
5.	*F. solani* v. *martii* ..	25·0	0·44

It will be seen from the mortality per cent in respect of egg-plant that all the isolates excepting isolate No. 5 caused death of plants to varying extent. Out of 30 isolates tested five viz., 6, 21, 22, 26 and 29 showed a range of 50 to 75 per cent and they were statistically on par. The remaining isolates showed mortality ranging from 25 to 37·5 per cent and all of them were statistically on par. Out of 29 pathogenic isolates 18 were found to be *F. solani*, 10 belonged to *F. solani* v. *minus* and one isolate was found to be *F. solani* v. *martii*. It was also observed that all the isolates caused rotting of the tap and lateral roots of the surviving plants in varying degrees. The virulence of the isolates has, therefore, to be compared from two angles viz., percentage of mortality and root rot index. The results in table 5 reveal that in general isolates exhibiting high mortality have shown low root rot indices in comparison with root rot indices of the isolates of low mortality. It is thus seen that there was a certain amount of inverse correlation between mortality and root rot index.

The results in respect of chilli isolates show that out of 35 isolates tested three isolates (Nos. 1, 8 and 35) were found to be non pathogenic. Six isolates showed high virulence causing 100 percent mortality and they were statistically on par. Four isolates caused 80 per cent mortality. Eleven isolates showed a mortality range of 50 to 60 per cent. The remaining isolates were found to show mortality ranging from 20 to 40 per cent. It was further observed that out of 32 pahogenic isolates 14 were found to be *F. solani*, 14 belonged to *F. solani* v. *minus* and 4 were found to

be *F. solani* v. *martii*. Both *F. solani* and *F. solani* v. *minus* appeared to exist in strains of differing pathogenic ability.

The results in respect of tomato isolates showed that out of six isolates tested one (No. 2) was found to be non pathogenic while the rest of the isolates were weakly pathogenic on tomato. It was also observed that all the isolates were found to be *F. solani* v. *minus* only.

Cross inoculation studies:

The isolation of causal organisms from diseased specimens collected in different districts of the Madras State revealed the presence of the same varieties and species of *Fusarium* on all the three Solanaceous hosts viz., egg-plant, chilli and tomato. With a view to ascertain whether the isolates of *Fusarium* from one host could pass on to other Solanaceous host plants, cross inoculation studies were conducted with few selected isolates that were found to be virulent on their own respective hosts.

The inoculation tests were carried out as per the method already described. The mortality of plants under each isolate was recorded and the percentage mortality was used as the criterion for assessing the cross-pathogenic ability of the various isolates. The data obtained with isolates from egg-plant, chilli and tomato on all the three hosts are given in table 6.

TABLE 6

Mean mortality per cent of isolates of Fusarium spp. from egg-plant on chilli and tomato

Isolate number (1)	Var. and sp. of *Fusarium* (2)	Egg-plant (3)	Chilli (4)	Tomato (5)
6	*F. solani* ..	70	40	30
8	" ..	20	80	80
15	" ..	50	80	20
17	" ..	30	90	40
27	" ..	60	80	80
29	" ..	60	70	80
2	*F. solani* v. *minus* ..	30	50	20
26	" ..	30	30	70
14	*F. solani* v. *martii* ..	10	50	80

Isolate number (1)	Var and sp. of *Fusarium* (2)	Egg-plant (3)	Chilli (4)	Tomato (5)
	Isolates from chilli on egg-plant and tomato			
12	*F. solani* ..	0	50	60
18	„ ..	30	90	20
23	„ ..	20	20	20
32	„ ..	20	40	50
34	„ ..	20	50	10
6	*F. solani* v. *minus* ..	30	40	10
7	„ ..	30	30	0
16	„ ..	20	90	20
20	„ ..	30	20	30
27	„ ..	0	20	0
	Isolates from tomato on egg-plant and chilli			
1	*F. solani* v. *minus* ..	0	0	20
2	„ ..	0	0	0
3	„ ..	10	10	10
4	„ ..	0	10	20
5	„ ..	0	10	10
6	„ ..	10	10	10

The results in respect of isolates from egg-plant indicate that all of them are able to infect chilli and tomato besides their own host viz., egg-plant. The data were statistically analysed and found to be significant. It was observed that chilli was the most susceptible host followed by tomato. Egg-plant which is their own host was least susceptible to all the isolates tested. The interaction between hosts and the isolates reveal that high mortality was recorded with isolate Nos. 27, 6 and 29 in the case of egg-plant, isolate Nos. 17, 8, 15 and 27 in the case of chilli and isolate Nos. 8, 14, 27, 26 and 29 in the case of tomato. It is, therefore, clear that the isolates causing maximum mortality in each crop vary indicating thereby that virulence is a specific character with reference to particular host.

The results in respect of chilli isolates on egg-plant and tomato reveal that most of the isolates tested were found to infect egg-plant and tomato besides chilli which is their own host. The data were found to be statistically significant. The results further showed that chilli was the most highly susceptible host, egg-plant and tomato being next and on par. Isolate Nos. 16 and 18 record-

ed highest infection on chilli and are on par. Isolate No. 27 has failed to infect both egg-plant and tomato. Isolate No. 12 has failed to infect egg-plant while isolate No. 7 has failed to infect tomato. The results of interaction between crops and isolates have revealed that eight isolates (Nos. 23, 6, 7, 18, 20, 16, 32 and 34) are on par in the case of egg-plant, four isolates (Nos. 12, 32, 23 and 20) are on par in the case of tomato and only two isolates (Nos. 16 and 18) are on par in the case of chilli. It is, therefore, clear that in the case of chilli isolates also maximum mortality in each crop was caused by different isolates indicating that the virulence of isolates is specific in respect of each host.

The results of cross-inoculations with isolates from tomato on egg-plant and chilli revealed that all of them are weak pathogens in comparison with isolates from egg-plant and chilli. Isolate No. 2 was found to be non pathogenic on all the three hosts. Isolate No. 1 was pathogenic only on tomato, isolate Nos. 4 and 5 were pathogenic on chilli and tomato and isolate Nos. 2 and 6 were pathogenic on all the three hosts. The results thus indicate that although most of the isolates from tomato are weakly pathogenic on these hosts they still caused mortality of plants showing thereby that they are cross-pathogenic.

Morphological characters:

The morphology of the different types of spores produced by various isolates was studied. All the isolates produced microconidia, macroconidia and chlamydospores. The microconidia were found either scattered in the aerial mycelium or in false heads. The shape of the microconidia was found to vary usually oval, oblong or kidney shaped; one to two celled but mostly one celled. The macroconidia were produced either in sporodochia or in aerial mycelium. They were found to be thick walled with distinct septa, the septation ranged from 0-4 but mostly 3. Macroconidia were abundant when produced on sporodochia, spindle to sickle shaped with pronounced curvature at the tip than in the middle of the spore. The apex was blunt or rounded. The chlamydospores were abundant, terminal or intercalary, one to two celled, in chains or clusters, smooth or rough walled.

Cultural characters:

The cultural characters like the amount of aerial mycelium its presence or absence, colour, etc., were recorded. Most of the isolates produced aerial mycelium which was either fluffy and

abundant or scanty and adpressed to the medium. The colour of the mycelium was mostly found to be white but in some cases it was yellowish white or cream coloured. Many isolates produced sporodochia but considerable variation in the amount and appearance of the sporodochia produced was noticed among the various isolates. The sporodochia remained embedded in the mycelium. Some isolates produced masses of macroconidia that appeared as wet cream or green coloured beads on the surface of the agar medium. Occasionally large patches of spore masses were evident when sporodochia were abundant. Sclerotia were absent in all the isolates studied.

The non sporodochial isolates produced aerial mycelium the nature and type of which was found to vary with different isolates. The aerial mycelium was coarse and ropy in some while it was fine and floccose in others. In certain non-sporodochial types the mycelium was sparse with no aerial growth and mostly adpressed to the medium.

It was observed that in general the characters exhibited by various isolates conformed very closely to those described by Wollenweber and Reinking (1935) for the Section *Martiella*. Within this Section the isolates were identified as *F. solani* and its two varieties viz., *F. solani* v. *minus* and *F. solani* v. *martii* on the basis of 3-septate macroconidial measurements. The following are the mean measurements of length and breadth for *F. solani* and its two varieties as given by Wollenweber and Reinking (1935):

F. solani	36 × 5·5 μ
F. solani v. *minus*	30 × 4·5 μ
F. solani v. *martii*	44 × 5·2 μ

The number of isolates from each host identified as *F. solani* and its two varieties are given in Table 7.

TABLE 7

No.	Isolate from	Number of isolates of			
		F. solani	*F. s* v. *minus*	*F. s* v. *martii*	Total
1.	Egg-plant ..	19	10	1	30
2.	Chilli ..	16	15	4	35
3.	Tomato ..	—	6	—	6

A careful analytical study of the cultural characters of the various isolates revealed that the variety and species of *Fusarium* could be classified into four different clonal types viz., abundantly sporodochial, sparsely sporodochial, abundantly mycelial and scantily mycelial. The morphological, cultural and pathogenicity characteristics of isolates of *Fusarium* from different hosts are summarized in Table 8.

Discussion

Frequent reports of wilting in egg-plant, chilli and tomato due to species of *Fusarium* from different parts of the Madras State prompted the author to make a detailed investigation of the problem. The association of *Fusarium* spp. with wilts of these crops have been reported earlier in the Madras State (Anon., 1954). But the exact disease syndrome has not been described. The specific identity of the causal Fusaria associated with the disease have also not been established. It became evident at an early stage of the investigation that the plants suffered from root rot and not true wilt. Varma (1954) has also shown that the so called wilt of tomato in this State was more of the nature of root rot than wilt of the vascular type. It may be pointed out here that species belonging to the Section *Martiella* are known to cause root rots and not vascular wilts (Vasudeva, 1935; Williams *et al.*, 1940; Gordon and Sprague, 1941; Reinking, 1950; Snyder *et al.*, 1959).

Numerous isolations made from the diseased roots yielded species of *Fusarium* which on detailed examination proved to belong to the Section *Martiella* of Wollenweber and Reinking (1935). Three members of this Section were encountered but only two of them viz., *F. solani* and *F. solani* v. *minus* were most frequently isolated while *F. solani* v. *martii* was encountered only very infrequently. The two former fungi were distributed very widely in the State. Varma (1954) while studying the tomato wilt Fusaria from the Madras State obtained several species of *Fusarium* belonging to many other Sections. However, 15 out of 37 isolates studied by him belonged to *Martiella* Section (*F. solani* v. *minus* and *F. solani* v. *martii*). In the present investigation while egg-plant and chilli yielded *F. solani*, *F. solani* v. *minus* and *F. solani* v. *martii* tomato yielded only *F. solani* v. *minus*. It must, however, be mentioned that only 6 isolates of tomato were studied in the present investigation.

The pathogenicity studies revealed that most of the isolates were pathogenic on their own host in varying degrees. Few isolates from each host were also found to be non-pathogenic. One isolate (No. 5) out of 30 isolates from egg-plant, three isolates (Nos. 1, 8, and 35) out of 35 isolates from chilli and one isolate (No. 2) out of 6 isolates from tomato were found to be non pathogenic. The pathogenic isolates of both egg-plant and chilli exhibited wide variations of high and low virulence with transitional forms in between these two extremes. The chillli host was found to be more susceptible to the isolates than egg-plant and tomato. Most of the tomato isolates were found to be only mildly pathogenic. Snyder *et al.* (1959) have put forth evidence to show that *Fusarium* root rot of bean incited by *F. solani* f. *phaseoli* was usually the result of multiple infection of the underground parts in the field by many clones. Venkataram (1955) studied pathogenicity of two isolates of *F. solani* and found mixed infections to be higher than the infection produced by either of the isolates singly. In the present study also several clonal types were often isolated from the same field and it is, therefore, likely that such multiple infections occur in nature.

In this investigation it was observed that the pathogen had two distinct effects on the host (egg-plant) viz., quick killing of the seedlings soon after transplanting in the infested soil and a slow continuing rotting of the roots resulting in stunting of the plants. In most root rot diseases the mortality of plants expressed as a percentage is taken as the criterion for assessing comparative virulence. However, in the present study it was observed that if the seedlings survived the initial onslaught of the pathogen, they continue to remain alive but in a very stunted and sickly condition. Such plants exhibited considerable decay of a large part of the tap root together with loss of a large part of the lateral roots. The rotting of the laterals appeared to be a continuing process. The stunting of the plants was invariably due to the extensive rotting of the root system. It was obviously difficult to overlook this effect of the pathogen in assessing the comparative pathogenicity of the isolates. A system of 'root rot index' was devised for the purpose of quantitative assessment of root rot. The root rot index is defined here as the ratio of the mean weight of diseased roots to the mean weight of roots of healthy plants of the same age and of the same variety and grown under identical conditions. Attempts have been made to express quantitatively the

extent of infection in root rot diseases. Mc Kinney (1923) developed an 'infection rating' which was also adopted by later workers, to assess the extent of disease in root rot of wheat caused by *Helminthosporium sativum*. In this 'infection rating' a serious drawback is that the extent of disease in each plant has to be arbitrarily determined by visual observation only. This will give room for a great deal of personal error. The 'root rot index' developed in the present study makes the assessment of extent of disease absolutely objective without any room for personal error. It can be used both for quantitatively expressing the comparative virulence of different isolates of a pathogen or the comparative resistance or susceptibility of a host variety.

In any assessment of pathogenicity of root rot fungi, therefore, it seems necessary to take into account these two independent aspects of the disease viz., mortality per cent and root rot index as otherwise misleading conclusions are likely to be drawn. The precise conditions under which these two aspects of pathogenicity are exhibited are not, however, clear An examination of the data on the mortality per cent and root rot index in Table 5 revealed that there was a certain amount of inverse correlation between mortality and root rot index. The isolates causing low death rate had generally a high mean root rot index. But certain isolates which caused low death rate had also very low root rot indices.

The cross inoculation studies carried out with selected isolates on three Solanaceous hosts viz., egg-plant, chilli and tomato have indicated that the different varieties and species of *Fusarium* causing root rot disease on these crops are cross pathogenic. The isolates causing high mortality were found to vary with the crop indicating that the virulence of isolates is specific with reference to particular host. The cross inoculation studies have also revealed that among the three hosts tested chilli was found to be the most highly susceptible followed by tomato. Egg-plant was found to be least susceptible to root rot disease. Venkatram (1955) studied pathogenicity of 75 isolates of *F. solani* on cotton and pigeon pea and found that in general *F. solani* cultures were more virulent on cotton than on pigeon pea.

Detailed investigations on morphological and cultural characters of the various isolates from three different hosts have revealed that all the isolates exhibited considerable variation in respect of these characters. Cultural variation in *Fusarium* species and

their resultant complication in the problem of taxonomy have been well recognised by several workers (Padwick, 1940; Snyder and Hansen, 1941; Miller, 1945, 1946a, 1946b; Subramanian, 1951). Among the morphological characters of the isolates studied, shape of the spore was found variable within the same isolate and was, therefore, not of much value in species determination. However, the length of the 3-septate conidium appeared to be a useful criterion in classifying the isolates. Three length groups could be recognised in the 3-septate conidia viz. 32 μ (20-40), 36 μ (24-44) μ and 40 μ (27-47). These corresponded to *F. solani* v. *minus*, *F. solani* and *F. solani* v. *martii* of Wollenweber and Reinking (1935). These lengths remained constant through several rounds of subculturing over a period of nearly 4 years. Isolates belonging to these three length groups were readily distinguishable even on casual observation under the microscope. It was, therefor, difficult to put isolates of these groups under one name viz., *F. solani* as advocated by Snyder and Hansen (1941). Wollenweber and Reinking (1935) have placed great reliance on the median diameter of 3 and 5-septate conidia and very minute differences in the order of 1 μ have been employed for species separation. It may be pointed out that the error in measurement may often exceed this limit and this criterion is, therefore, of very doubtful value.

The usefulness of morphological features in the identification of species has also been emphasised by Subramanian (1955). He found that several isolates of *Fusarium udum* showed constant morphological features although a wide range of variation was observed in cultural characters. The same was found to be the case with Fusaria obtained from *Gladiolus*. It was, therefore, felt that primary importance should be given to morphological characters especially the length of macroconidia in species separation of this genus. This should, of course, be supplemented with other characters like shape, nature of septation etc., of the conidia.

The cultural characters and pathogenic ability of the isolates to a large extent were found to be variable and could not be used as criteria for identifying the isolates. Most of the isolates exhibited considerable variation in cultural characters such as aerial mycelium, colony colour, presence or absence of sporodochia and pionnotes, relative abundance of conidia etc. Similar variation was observed by Varma (1954) also in *F. solani* and other species of *Fusarium* inciting root rot of tomato. It will be, therefore,

hazardous to base a system of classification on cultural characters alone. In this context the usefulness of the suggested catalogue of *Fusarium* clones by Waite and Stover (1959) also requires further examination. Snyder *et al.* (1959) have reported the existence of distinct pathogenic clones in *F. solani* f. *phaseoli,* inciting root rot of beans, on the basis of correlation between cultural characters and pathogenicity. In the present study, *F. solani* and *F. solani* v. *minus* were found to exist in 4 cultural types viz., abundantly sporodochial, sparsely sporodochial, abundantly mycelial and scantily mycelial. But the results have not indicated any correlation between cultural characters and pathogenicity as observed by Snyder *et al.* (1959). Varma (1954) has also reported that comparison of pathogenicity and growth characters in pure culture of all the isolates showed that there is no consistent correlation between the ability to produce wilt and growth characters.

The trinomial system of classification proposed by Snyder and Hansen (1940) was mainly based on pathogenicity. In the present investigation as well as in studies by many other workers (Subramanian, 1951; Venkataram, 1955) pathogenicity was found to be a widely varying character. Furthermore, there are also non pathogenic isolates in the same species. Under these circumstances it would be superfluous to accord any taxonomic status to the forms on the basis of pathogenicity to one or more hosts. Reviewing the overall situation it appears that only morphological characters like shape, size, septation and type of spores produced by the isolates could be used as criteria for species sepation in the genus *Fusarium.*

Summary

The root rot diseases caused by species of *Fusarium* on three important Solanaceous crops viz., egg-plant, chilli and tomato prevalent in the Madras State were investigated. These diseases were found to be widely distributed in all places wherever these crops are cultivated. The diseases affecting the three crops were found to be root rots and not vascular wilts. The causal organisms inciting the diseases were found to be *F. solani* and its two varieties viz., *F. solani* v. *minus* and *F. solani* v. *martii. F. solani* occurred in greater abundance than *F. solani* v. *minus* in the case of egg-plant while in chilli both the fungi occurred in equal abundance. In tomato only *F. solani* v. *minus* was found to be associated with the disease. The ubiquitous distribution of the mem-

bers of the Section *Martiella* of the genus *Fusarium* in the arable soils of different districts of the Madras State is noteworthy. All the isolates produced macroconidia which were 0-4 septate but mostly 3-septate. The isolates were identified based on their morphological characters and on the length of 3-septate macroconidia. Three length groups could be recognised in the 3-septate conidia viz., 32 μ (20-40), 36 μ (24-44) and 40 μ (27-47). There was no correlation between pathogenicity and cultural characters of the various isolates studied.

Pathogenicity tests carried out revealed that 29 out of 30 isolates of egg-plant, 32 out of 35 isolates of chilli and 5 out of 6 isolates from tomato were found to be pathogenic to varying extent on their respective hosts. The pathogenicity of *F. solani* and *F. solani* v. *minus* ranged from 25-50 and 25-75 per cent respectively in egg-plant. The pathogenicity of both these fungi ranged from 20-100 per cent in the case of chilli. In tomato it ranged from 10-20 per cent. The pathogenicity of *F. solani* v. *martii* was 25 per cent and 20-80 per cent in egg-plant and chilli respectively.

The isolates exhibited pathogenicity in two ways viz., a quick killing of the transplanted seedlings and a slow and continuous root rot resulting in the stunting of the affected plants. A 'root rot index' was devised so as to make a quantitative assessment of the extent of disease in egg-plant. There was a certain amount of correlation between mortality and 'root rot index.'

The cross inoculation tests have shown that the isolates of *Fusarium* from one host could infect the other two Solanaceous hosts. Among the three hosts tested susceptibility to *Fusarium* isolates were chilli > tomato > egg-plant.

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TABLE 8

Comparative morphological, cultural and pathogenicity characteristics of isolates of *Fusarium* from egg-plant, chilli and tomato

Isolate No.	var. and sp of *Fusarium*	Mycelium		3-septate conidia		Clonal type	Patho-genicity (Percent)	Root-rot index
		Nature of growth	Colour in rice meal	Mean (L×B) in μ	Range (L×B) in μ			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
	(A) *EGG-PLANT*							
1.	*F. solani*	Scanty	dirty white	35·1 × 4·2	32 2—37·8 × 3·5— 4·9	M	37·5	0·25
2.	*F.* s. v. *minus*	„	9 D_2 (Cream)	33·7 × 4·4	30·8—35·8 × 4 2— 4·9	M	25·0	0·50
3.	*F. solani*	Aerial Myc. scanty	1 B 7 (Pink $_1{}^T$)	37·8 × 4·9	36 4—42 × 4·2— 5·6	SS	25·0	0·06
4.	*F.* s. v. *minus*	Fairly abundant	1 B 7 (Pink 1^T)	32·2 × 4·2	28·0—32·8 × 4·2— 4·9	SS	37·5	0·11
5.	*F. solani*	Scanty	6 C 9 (Rose stone)	35·1 × 4·4	28·0—39·2 × 4·2— 4·9	M	0·0	0·69
6	„	„	9 B 2 (Polar bear)	35·1 × 4·7	32·2—37·8 × 4·2— 5·6	S	50·0	0·44
7.	„	Abundant & fluffy	11 B 1 (New silver)	35·5 × 4·2	29 4—44·8 × 4·2	MM	25·0	0·03
8.	„	Scanty	1 B 7 (Pink 1^T)	37·8 × 4·9	28 0—40·6 × 4·2— 5·6	M	25·0	0·67
9.	*F.* s. v. *minus*	Not abundant	9 D 2 (Cream)	31·7 × 4·2	28 0—35·0 × 4 2	M	25·0	0·83

Isolate No.	var. and sp. of *Fusarium*	Mycelium		3-septate conidia		Clonal type	Pathogenicity (Percent)	Root-rot index
		Nature of growth	Colour in rice meal	Mean (L×B) in μ	Range (L×B) in μ			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
10.	*F. solani*	Scanty	10 B 6 Sunrise	39·2 × 4·7	28·0—43·4 × 4·2— 4·9	M	25·0	0·92
11.	*F. solani*	Fairly abundant	11 B 4 (nude season)	34·0 × 4·2	28·0—39·2 × 4·2— 5·6	MM	25·0	0·36
12.	„	Scanty	2 B 1	35·0 × 4·7	28·0—39·2 × 4·2— 5·6	SS	25·0	0·39
13.	*F.* s. v. *minus*	Not abundant	11 F 6	28·0 × 4·2	25 2—32·2 × 4·2— 4·2	S	37·5	0·36
14.	*F.* s. v. *martii*	Abundant & fluffy	417 (Pomogranate Pr)	44·6 × 5·2	39·2—47·6 × 4·2— 5·6	SS	25·0	0·44
15.	*F. solani*	Scanty	42 A 3	35·0 × 4·9	30·8—39·2 × 4·2— 5·6	S	37·5	0·22
16.	*F.* s. v. *minus*	„	10 C 3 (Vanilla)	38·9 × 5·2	36·4—39·2 × 4·2— 5·6	M	25·0	0·31
17.	„	Abundant & fluffy	44 B 1 (Shadow)	36·4 × 5·2	32 2—42·0 × 4·2— 5·6	MM	25·0	0·53
18.	*F.* s. v. *minus*	Scanty	3 E 7 (Power-PK)	36·4 — 4·4	32·2—39·2 × 4·2— 4·9	M	37·5	0·28
19.	*F.* s. c. *minus*	„	3 B 9 (Rose dawn)	33·0 × 4·2	30·8—35·0 × 4·2	S	25·0	0·31
20.	„	Fairly abundant	9 D 2 (Cream)	32·2 × 4·2	28·0—35·0 × 4·2— 4·9	S	37·5	0·28
21.	„		53 B 1 (Mist)	32·2 × 4·4	26·6—40·6 × 4·2— 4·9	S	75·0	0·11

22.	„	Scanty	10 F 2 (Straw)	32·2 × 4·4	29·4—35 × 4·2— 4·9	M	62·5	0·14
23	*F. solani*	„	11 D 7 (golden wheat)	35·0 × 4·9	30·8—37·8 × 4·2— 5·6	M	25·0	0·50
24.	„	„	3 B 7 (Iris maure)	35 0 × 4·9	29·4—37·8 × 4·2— 5·6	M	37·5	0·22
25.	„	„	43 A 1 (Agate Gy+)	36·4 × 5·2	30·8—39·2 × 4·2— 5·6	SS	25·0	0·36
26.	*F.* s. v. *minus*	„	43 A 1 (Agate Gy+)	32·2 × 4·2	28·0—37 8 × 4·2	M	75·0	0·17
27.	*F. solani*	„	9 B 2 (Polar bear)	36 4 × 4·9	28·0—42·0 × 4·2— 5·6	S	50·0	0·28
28.	„	„	2 E 7 (Hydrangea)	39·6 × 5·2	36·4—42·0 × 4·2— 5·6	SS	25·0	0·42
29.	„	„	43 A 1 (Agate Gy+)	37 8 × 4·9	36·4—40·6 × 4·2— 5·6	M	25·0	0·83
30.	*F. s.* v. *minus*	„	43 B 4 (Plumbago blue)	32·2 × 4·2	28·0—35·0 × 3·5— 4·9	S	25·0	0·36
	(B) *CHILLI*							
1.	*F. solani*	„	White	35·0 × 4·6	28·0—37·8 × 4·5— 5·4	M	0	—
2.	F. s. v. *minus*	Abundant	„	30·4 × 4·6	23·6—34·6 × 4·5— 5·4	MM	20	—
3	„	„	„	30·9 × 4·7	25 5—38·2 × 3·6— 5·4	MM	40	—
4	F. s. v. *minus*	Abundant	„	30·6 × 4·5	25·5—36·4 × 3·6— 5·4	MM	60	—
5.	„	„	„	30·6 × 4·6	21·8—38·2 × 3·6— 5·4	MM	40	—

Isolate No.	var. and sp of *Fusarium*	Mycelium		3-septate conidia		Clonal type	Pathogenicity (Percent)	Root-rot index
		Nature of growth	Colour in rice meal	Mean (L×B) in μ	Range (L×B) in μ			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
6.	„	Scanty	White	28·7 × 4·9	26·8—34 6 × 4·5— 5·4	S	80	—
7.	„	Abundant	„	29·0 × 4·9	23·7—38·2 × 3·6— 5·4	MM	100	—
8.	*F. solani*	Scanty	„	31·3 × 4·5	20·0—40 × 3·6— 5·4	M	0	—
9.	*F.* s. v. *minus*	„	„	28·8 × 4·2	23·7—32·8 × 3·6— 4·5	S	60	—
10.	*F. solani*	„	„	33·8 × 5·2	24·0—39·6 × 4·5— 5·4	SS	60	—
11.	„	„	„	30·7 × 5·0	24·8—41·4 × 3·6— 5·4	S	50	—
12.	„	„	„	34·6 × 4·6	26·7—40·0 × 4·5— 5·4	S	100	—
13.	*F.* s. v. *minus*	Abundant	„	28·9 × 4·5	20·0—36·4 × 3·6— 5·4	S	40	—
14.	*F.* s. v. *martii*	„	„	37·1 — 4·9	27·3—47·3 × 4·5— 5·4	S	20	—
15.	*F. solani*	Scanty	„	33·3 × 4·9	30·9—40·7 × 3·6— 5·4	M	40	—
16.	*F.* s. v. *minus*	Abundant	„	32·4 × 4·4	27·3—38·2 × 3·6— 5·4	MM	80	—
17.	*F. solani*	Scanty	„	34 7 × 5·1	28·8—40·4 × 3·6— 5·4	M	40	—

18.	*F.* s. v. *martii*	„	„	39·0 × 4·9	30·9—45·5 × 4·5— 5·4	M	80	—
19.	*F. solani*	„	„	37·8 × 5·2	32·8—40 × 4·5— 5·4	S	60	—
20.	*F.* s. v. *minus*	„	„	30·9 × 4·1	27·3—36·4 × 3 6—5·4	S	100	—
21.	*F.* s. v. *martii*	Abundant	„	39·0 × 4·7	32·4—43·2 × 3·6— 5·4	S	60	—
22.	*F. solani*	Scanty	„	34·6 × 4·9	29·1—40 × 3·6— 5·4	M	60	—
23.	*F. solani*	„	„	36·3 × 5·5	32·6×40 × 4·5— 5·4	S	80	—
24.	*F.* s. c. *minus*	„	„	31·2 × 4·9	27·3—38·2 × 3·6— 5·4	M	40	—
25.	*F. solani*	„	„	36·9 × 5·7	27·3—43·7 × 4·5— 5·4	S	60	—
26.	*F. solani*	„	„	36·8 × 5·3	32·8—43·2 × 4·5— 6·3	M	60	—
27.	*F.* s. v. *minus*	Abundant	„	32·0 × 4·6	27·3—38·2 × 3·6— 5·4	MM	100	—
28.	*F.* s. v. *martii*	Scanty	„	32·4 × 4·3	27·3—40·0 × 3·6— 5·4	M	60	—
29.	*F.* s. v. *martii*	„	„	38·0 × 5·0	30·9—45·5 × 4·5— 5·4	M	20	—
30.	*F.* s. v. *minus*	„	„	31·2 × 4·9	25·5—38·2 × 3·6— 5·4	S	60	—
31.	*F. solani*	„	„	34·2 × 5·2	28·8—38·6 × 3·6— 5·4	S	20	—
32	F. solani	„	„	38·4 × 4·8	30 9—44·5 × 3·6— 5·4	M	100	—

Isolate No.	var. and sp. of *Fusarium*	Mycelium		3-septate conidia		Clonal type	Patho-genicity (Percent)	Root-rot index
		Nature of growth	Colour in rice meal	Mean (L×B) in μ	Range (L×B) in μ			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
33.	*F. solani*	„	„	34·0 × 5·2	27·3—41·8 × 4·5— 6·3	S	40	—
34.	*F. solani*	„	„	34·6 × 5·2	28·8—41·0 × 4·5— 6·3	S	100	—
35.	*F. s.* v. *minus*	„	„	31·5 × 4·9	27·3—32·8 × 3·6— 5·4	S	0	—
	(C) *TOMATO*							
1.	*F. s.* v. *minus*	Abundant	„	33·6 × 4·2	28·0—37·8 × 4·2	MM	20·0	—
2.	„	Scanty	„	30·2 × 4·6	25·2—36·4 × 4·2— 4·9	M	0·0	—
3.	„	„	„	30·8 × 4·3	25·2—37·8 × 4·2— 4·9	M	10·0	—
4.	„	„	„	30·2 × 4·6	25·2—35·0 × 4·2— 4·9	M	20·0	—
5.	„	„	„	30·8 × 4·3	26·6—39·2 × 4·2— 4·9	M	10·0	—
6.	„	„	„	32·2 × 4·3	25·2—37·8 × 4·2 — 4·9	M	10·0	—

Note: SS—Sporodochia abundant; S—Sporodochia sparse; MM—Mycelium abundant; and M—Mycelium sparse

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J. Madras Univ., B, XXXIII, No. 3, pages 231-248, 1963

Some Calcareous Foraminifera belonging to the Families Rotaliidae, Globigerinidae, Globorotaliidae and Anomalinidae from the Cullygoody (Dalmiapuram) Limestone, Trichinopoly Cretaceous of South India

PART III

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Introduction:

This is the third in a series on the Cullygoody Foraminifera of the Trichinopoly Cretaceous. It is mainly concerned with the systematic descriptions of some trochoid foraminifera, which are grouped into four families namely Rotaliidae, Globigerinidae, Globorotaliidae and Anomalinidae. Of these, Rotaliidae is most varied and abundant representing 5 genera namely *Trocholina, Discorbis, Gyroidina, Parrella,* and *Rotalia.* Globigerinidae and Globorotaliidae are represented by *Globigerina* and *Globorotalites* respectively while *Anomalina* and *Cibicides* represent the family Anomalinidae.

In the following pages are described and figured 14 species and one variety of which two species namely *Rotalia cullygoodiensis* and *Trocholina raoii* are new. All the figured specimens are deposited provisionally in the Geology Department, University of Madras, Madras.

Systematic descriptions

Family Rotaliidae

Subfamily Turrispirillininae

Genus Trocholina paalzon, 1922

Trocholina raoii sp. nov.

Pl. 4, Figs. 11-13

The trochoid test is plano-convex with its conical dorsal and almost flattened ventral side. Externally all the whorls are visible on the dorsal side whereas only the last whorl is visible on the ventral. Only one tubular chamber of 8 to 10 coils in length gra-

dually increases in size towards its apertural end. ' In small specimens the distinct spiral sutures are flush with the surface but depressed in large specimens. On the ventral side the umbilicus is covered by the secondary shell material. The calcareous wall is typically green. On the dorsal side it is smooth but ornamented on the ventral side, with an inner circular row of pustules and an outer circular row of pillars. At the distal end the chamber opens out ventrally into a semi-circular aperture.

Remarks: This commonly occurring species resembles *T. lithographica* (Gumbel) reported from the Jurassic of Germany in its convexity of the dorsal surface but differs from the latter in having more number of whorls.

This species is named in honour of Prof. L. Rama Rao, who has done extensive research-work in this area.

Type-level:—Lower Cretaceons

Type locality:—Occurs abundantly in the Cullygoody limestone quarries.

Type specimens:—Provisionally deposited in the Geology Department of University of Madras.

Specimen Number:—The Register number will be given later.

Sub family Discorbinae

Genus *Discorbis* Lamarck, 1804

Discorbis minima Vieaux

Pl. 1, Figs. 10-13

Discorbis minima Vieaux, 1941, *J. Paleont*, Vol. 15, No. 6, p. 627, Pl. 85. Figs. 10 a-c.; *Discorbis* cf. *D. minima* Vieaux., Loeblich and Tappan, 1949, *J. Paleont.*, Vol. 23, p. 265, pl. 51, Figs. 12-13.

The longer than broad trochoid test is concavo-convex with its convex dorsal side and concave ventral side. It consists of about 2 whorls which are visible on the dorsal side but only the last whorl is visible on the ventral side. A depressed umbilicus is present on the ventral side. In the earlier whorl, the chambers are small and rather distinct whereas in the later whorl, they are about 6 to 7 in number, rapidly increasing in size, more inflated on the dorsal side than on the ventral and broader at the outer margins than at the inner. The sutures of the earlier whorl are indistinct but those of the latter whorl are distinct, depressed and oblique. The periphery is acutely angled. The finely perforate

calcareous wall is smooth and sub-transparent. On the ventral side, a long curved aperture occurs at the inner margins of the last two chambers.

Remarks: A few specimens occurring in my material are similar to the figures of *D. minima* reported by Vieaux and Loeblich and Tappan from the Lower Cretaceous of America.

Discorbis sp.
Pl. 3, Figs. 14-16

Description: The subcircular trochoid test is biconvex. Usually it is almost equally biconvex but rarely, its dorsal side is more convex than the ventral. It consists of about 2½ whorls. Externally all the whorls are visible on the dorsal side but only the last whorl is visible on the ventral. A depressed umbilicus occurs on the ventral side. In the earlier whorls, the chambers are indistinct but distinct when wet. In the final whorl, they are distinct, 5 in number, inflated and rapidly increasing in size. On the dorsal side, they are elliptical in shape, less inflated and much broader than high whereas on the ventral side, they are triangular with their bases at the periphery and pointed ends at the umbilicus, more inflated and higher than broad. In the earlier whorls, the sutures are indistinct but in the final whorl, they are distinct and much depressed. On the dorsal side, they are oblique but straight on the ventral. The broadly rounded periphery is lobulate The calcareous wall is cancellated but sometimes smooth and polished. The slit-like aperture occurs near the umbilicus at the base of the apertural face.

Remarks: Such specimens are commonly found in my material.

Genus *Gyroidina d'Orbigny*, 1826
Gyroidina loetterlei Tappan
Pl. 1, Figs. 4-6

Gyroidina loetterlei Tappan, 1940, *J. Paleont.*, Vol. 14, p. 120, Pl. 19, Figs. 10 a-c; *Ibid*, 1943, Vol. 17, p. 512, Pl. 82, Figs. 9 a-c.

The sub-circular trochoid test is plano-convex with an almost flattened dorsal side and a convex ventral side and consists of about 1½ to 2 whorls. Dorsally all the whorls are visible but ventrally only the last whorl is visible. The chambers of the last whorl are about 6 to 7 in number, increasing more rapidly in size than those of the earlier whorls and the last two to three are

inflated. On the dorsal side, the chambers are as broad as high and slightly broader at the outer margins than at the inner. On the ventral side, they are triangular with their bases at the outer margins and pointed ends at the depressed umbilicus. The sutures of the earlier whorls are indistinct but those of the final whorls are distinct and depressed. On the dorsal side, the spiral suture is depressed. The periphery is broadly rounded and lobulate. The calcareous smooth wall is polished. A long narrow slit-like aperture with a thin lip occurs at the base of the apertural face between the periphery and the umbilicus.

Remarks: My commonly occurring specimens are identical with the description and figures of Tappan's species reported from the Grayson and Duck Creek Formations of Texas and Oklahoma.

Gyroidina globosa (Hagenow)
Pl. 1, Figs. 1-3

Nonionina globosa Hagenow, Neues. Jahrb., 1842, p. 574. *Rotalia globosa* Reuss, 1862, K. Akad. Wiss. Wien., Math. Naturwiss. Kl. Sitzungsber, Vol. 44, Pt. 1, p. 330, Pl. 7, Figs. 2 a-b.

Gyroidina globosa (Hagenow) Cushman, 1946, U.S. Geol. Surv. Prof. Paper 206, p. 140, Pl. 58, Figs. 6-8.

The plano-convex trochoid test is subcircular in top-view with a flattened evolute dorsal side and a convex involute ventral side and consists of about two whorls. Sometimes the earlier whorls are slightly raised above the final whorl. The chambers of the earlier whorl are indistinct but those of the final whorl are distinct, about 7 in number and rather gradually increasing in size. On the dorsal side, they are narrow and higher than broad and subrectangular whereas on the ventral side, they are much broader than high and triangular with their broad outer margins and pointed inner margins. The sutures are indistinct except the last few which are depressed. On the dorsal side the spiral suture is depressed. The calcareous smooth wall is polished. On the ventral side, a depressed umbilicus is present. The periphery is rounded. Between the periphery and the umbilicus, a long narrow slit-like aperture with a distinct lip occurs on the ventral side at the base of the elliptical apertural face.

Remarks: My abundantly occurring specimens are identical with G. *globosa* described by Hagenew and figured by Reuss & Cushman.

This species closely resembles *G. loetterlei* Tappan but differs in having a more convex ventral side, uninflated and dorsally narrower chambers, less depressed sutures and in having less broadly rounded periphery.

Subfamily Rotaliinae
Genus Rotalia Lamarck, 1804
Rotalia umbonella Reuss.
Pl. 2, Figs. 1-4

Rotalia umbonella Reuss, 1860, K. Akad. Wiss. Wien, Math.-Naturw. Cl. Sitzber, Wien., Osterreich, Bd. 40, p. 221, Pl. 11, Fig. 5 a-c.

The subcircular trochoid test is plano-convex with a convex dorsal side and a plane ventral side. Dorsally the initial whorls are indistinct and appear as a central raised boss or umbo. Ventrally only the last whorl is visible and has an umbilicus covered by a solid plug. Variation occurs in number and size of the chambers of the final whorl. In the final whorl of small specimens, the arcuate rectangular chambers are 7 to 8 in number and increasing rapidly in breadth but gradually in height, whereas in large specimens, they are about 10 to 12 in number, increasing more rapidly in breadth and flaring. In the earlier whorls, the sutures are indistinct but in the later whorls, they are curved backwards, limbate and flush with the surface except the last few which are raised. The periphery is acutely rounded. The calcareous smooth wall is often covered by a fine secondary shell-material. On the ventral side the curved slit-like aperture occurs at the base of the end chamber near the periphery.

Remarks: The raised boss-like early chambers, the flaring later chambers, the distinct plug on the umbilicus and the backwardly curved limbate sutures are the distinguishing characters of this species.

My abundantly occurring specimens are similar to *R. umbonella* Reuss.

Rotalia cullygoodiensis sp. nov.
Pl. 2, Figs. 5-7

Truncatulina falcata Chapman (not Reuss), Quart. J. Geol. Soc., Vol. 50, pp. 721-2, Pl. 34, Figs. 15 a-c.

The subcircular trochoid test is plano-convex with a conical dorsal side and a plane ventral side. It consists of about 2 to 3 rather distinct whorls. Dorsally all the whorls are visible but ventrally only the last whorl is visible and has an umbilicus covered by a solid plug. The chambers of the earlier whorl are indistinct but those of the last whorl are more visible on the ventral side than on the dorsal. On the dorsal side, the gradually increasing chambers are sub-rectangular and higher than broad whereas on the ventral side, they are triangular with their bases at the periphery and their thickened pointed ends at the umbilicus and broader than high except the last chamber which is as high as broad. The sutures are indistinct except the last few. Dorsally they are oblique but slightly arcuate and depressed ventrally. The periphery is acutely angled. The smooth calcareous wall is covered by a fine secondary shell material making the chambers and sutures obscure. On the ventral side, a long slit-like aperture occurs at the base of the final chamber between the periphery and the umbilicus.

Remarks: My abundantly occurring specimens are similar to the figures of Chapman who has wrongly identified his species from the Gault of Folkestone as *Truncatulina falcata* Reuss. However it is found on comparison that my specimens and Chapman's figures differ considerably from the species of Reuss reported from the Oligocene. The latter is evolute both dorsally and ventrally and has no plug.

This species is similar to *R. umbonella* Reuss but differs in having a more conical dorsal side, higher than broad chambers dorsally and in the absence of much arcuate limbate sutures.

Type-level: Lower Cretaceous.

Type locality: Occurs abundantly in the Culygoody Limestone quarries.

Type specimens: Provisionally deposited in the Geology. Department of University of Madras.

Specimen number: The Register number will be given later.

Genus *Parrella* Finlay, 1939

Parrella navarroana (Cushman)

Pl. 3, Figs. 7-9

Pulvinulinella navarroana Cushman, 1938, contr. Cushman Lab. Foram. Res., Vol. 14, p. 66, Pl. 11, Fig. 5.

Pseudoparrella navarroana (Cushman), Fizzell, B. Econ. Geol., Univ., Texas, Austin, Rept. Invest. 22, p. 126, Pl. 19, Figs. 16 a-c.

The suboval trochoid test is equally biconvex and consists of about 2 whorls. On the dorsal side, the chambers of all the whorls are visible but on the ventral side, only those of the final whorl, which are about 10 to 12 in number, are visible. The sub-rectangular chambers are broader than high, oblique and gradually increasing in size. The limbate sutures are flush with the surface and curve backwards. The acutely angled periphery is keeled. The sub-transparent calcareous wall is smooth, polished and rather coarsely perforate. Ventrally, the umbilical area is closed with a solid mass of shell material. On the ventral side, a narrow slit-like aperture occurs at the base of the triangular apertural face and extends at a distinct angle into the apertural face.

Remarks: Since this species has the umbilicus covered with a solid mass of shell material and the aperture being extended at a distinct angle into the apertural face, it is included under the genus *Parrella*.

The figures of *P. navarroana* (Cushman) are similar to my commonly occurring specimens.

Family Globigerinidae
Subfamily Globigerininae
Genus *Globigerina* d'Orbigny, 1826
Globigerina cretacea d'Orbigny
Pl. 4, Figs. 1-4

Globigerina cretacea d'Orbigny, 1840, *Mem. Soc. Geol.* France, Paris, Ser. 1. to 4, p. 34, Pl. 3, Figs. 12-14; *Ibid,* Tappan, 1943; *J. Paleont*, Vol. 17, p. 512, Pl. 82, Figs. 16-17.

The suboval trochoid test is concavo-convex with the convex dorsal side and the concave ventral side and consists of about 2 whorls. Dorsally all the whorls are visible but ventrally only the final whorl is visible. The globular chambers of the initial whorl are rather distinct and small, whereas those of the final whorl are distinct, 5 in number, large and rapidly increasing in size and inflation particularly the end chamber. The distinct sutures are deeply excavated. The broadly rounded periphery is lobulate. The calcareous wall is cancellated. On the ventral side, a depressed umbilicus is present. At the base of the end chamber, the aperture opens out ventrally into the umbilicus.

Remarks: The specimens which occur quite abundantly in my samples are identical with *G. cretacea* d'Orbigny.

Globigerina cretacea var. *plana* Schacko
Pl. 3, Figs. 4-6

Globigerina cretacea var. *plana* Schacko, 1897, Ver. Treunde Naturg. Mecklenburg, Archiv. Gunstrow Jahrg. 50 (1896), pp. 184, 288.

The suboval trochoid test is concavo-convex with the dorsal side being convex and the ventral side being concave and consists of about 1½ whorls. Dorsally all the whorls are visible but only the final whorl having 4 chambers is visible ventrally. The globular chambers are rapidly increasing in size and inflation particularly the end chamber which occupies about ⅓ of the size of the test. The distinct sutures are depressed. The lobulate periphery is broadly rounded. The calcareous wall is smooth and polished but not cancellated. On the ventral side, the depressed umbilicus is present and a semicircular plate is attached at the inner margin of the end chamber. On the ventral side, the aperture opens out from below the plate into the umbilicus.

Remarks: The distinguishing features of this species are its large end chamber occupying about ⅓ of the size of the test and the presence of a semicircular plate attached ventrally at the inner margin of the end chamber.

Such specimens are quite abundant in my material. They may be the same as *G. cretacea* var. *plana* Schacko

Globigerina planispira Tappan
Pl. 4, Figs. 8-10

Globigerina planispira Tappan, 1940, *J. Paleont*, Vol. 14, No. 2, p. 122, Pl. 19, Figs. 12 a-c.

The small circular test consists of 2 whorls and is subtrochoid with the final whorl being almost planispiral. On the dorsal side, all the chambers are visible but on the ventral side, only those of the final whorl are visible. They are globular, increasing gradually in size and inflation towards the apertural end and are about 7 in the final whorl. The distinct sutures are deeply excavated. The broadly rounded periphery is lobulate. The white calcareous wall is cancellated. On the ventral side, the umbilicus is often covered with fine secondary shell material. Between the

periphery and the umbilicus, a slit-like aperture occurs ventrally with a thin plate attached to the base of the end chamber.

Remarks: The figures of Tappan's species reported from the Grayson Formation, Texas, are identical with my abundantly occurring specimens.

Globigerina sp.
Pl. 4, Figs. 5-7

The small subtrochoid test is almost oval in shape, longer than broad and consists of 1½ whorls. On the dorsal side, all the whorls are visible but only the final whorl having 5 chambers is visible on the ventral side. The globular chambers are increasing rapidly in size and inflation. The distinct sutures are deeply excavated. The broadly rounded periphery is lobulate. On the ventral side, a deeply depressed umbilicus is present. The calcareous wall is cancellated. A spine-like projection occurs at the outer margin of the end chamber. Ventrally a large arch-shaped aperture occurs between the periphery and the umbilicus.

Remarks: The characteristic features of the species are the longer than broad sub-trochoid test, a large arch-shaped aperture occurring between the periphery and the umbilicus and the spine-like projection at the outer margin of the end chamber.

Only three but well preserved specimens are found in my samples.

Family Globorotaliidae
Genus *Globorotalites* Brotzen, 1942
Globorotalites michelniana (d'Orbigny)
Pl. 3, Figs. 1-3

Rotalina michelniana d'Orbigny 1840 *Mem. Soc. geol.* France. Ser. 1, Vol. 4, p. 31, Pl. 3, Figs. 1-3.

Eponides michelniana Plummer, 1931, Texas Univ., Bull. 3101, p. 192, Pl. 14, Fig. 11.

Globorotalia michelniana (D'Orbigny) Cushman, 1946. U. S. geol. surv. Prof. Paper 206, p. 152, Pl. 63, Figs. 2 a-c (not Figs. 3 a-c).

The plano-convex trochoid test is almost circular from top view and has a flattened dorsal side and a conical ventral side with a closed umbilicus. On the dorsal side, about 2 to 2½ whorls are

visible but on the ventral side only the last whorl is visible and consists of about 7 to 8 chambers. Dorsally the gradually increasing chambers are obliquely set, broader than high and broader at the outer margins than at the inner whereas ventrally they are triangular with their bases at the periphery and pointed ends at the umbilicus. On the dorsal side, the limbate sutures are strongly oblique and slightly raised but on the ventral side, they are straight and the last few become slightly depressed. The periphery is acutely angled with a thin keel. The sub-transparent finely perforate calcareous wall is smooth, and polished. Sometimes it is brownish red in the earlier whorls. The long curved slit like aperture occurs at the base of the apertural face between the periphery and the umbilicus.

Remarks: My commonly occurring specimens are identical with *G. michelinıana* (d'Orbigny). Cushman (1946) gave two types of Figures (2 a-c and 3 a-c). In one of his types (i.e. 2 a-c), the umbilicus is closed whereas in the other type (i.e. 3 a-c) it is open. However my specimens are exactly similar to the former.

Family Anomalinidae
Subfamily Anomalininae
Genus *Anomalina* d'Orbigny, 1826
Anomalina intermedia Berthelin
Pl. 3, Figs. 10-13

Anomalina intermedia Berthelin, 1880 *Mem. Soc. Geol.* France, Paris, Ser. 3, tome 1, No. 5, p. 67, Pl. 4, Figs. 14 a-c.

The almost oval subtrochoid test varies in convexity. Some are unequally biconvex and others are plano-convex. The dorsal side is always convex and evolute whereas the ventral side is either flat or less convex and partially involute. On the dorsal side, the earlier whorls often appear as a central raised boss but in some specimens they are depressed. The final whorl is slightly embracing the earlier whorls and consists of about 8 to 10 chambers. The chambers of the earlier whorls are indistinct but those of the final whorl are broader than high, broader at the outer margins than at the inner and gradually increasing in size and inflation. In the earlier whorls the sutures are indistinct but in the final whorl, they are distinct, depressed and curving backwards. On the dorsal side the spiral suture is much depressed. The periphery is angled. The smooth calcareous wall shows dull lustre. The low arch-shaped aperture is peripheral, extending

slightly towards the ventral side and occurs at the base of the sub-triangular apertural face.

Remarks: Such specimens are abundantly found in my samples and are similar to the figures of Berthelin's species reported from the Lower Cretaceous of France.

Subfamily Cibicidinae
Genus *Cibicides* Montfort, 1808
Cibicides sandidgei Brotzen
Pl. 1, Figs. 7-9

Cibicides sandidgei Brotzen, 1936, Sver. Geol. Unders. Avh., Stockholm, Ser.C., No. 396 (Arsb. 30), p. 191, Pl. 14, Figs. 3-4 (not Fig. 2).

The oval trochoid test is plano-convex with a flattened dorsal side and a convex ventral side having a deep narrow umbilicus. On the dorsal side, the chambers of the earlier whorls are covered by the extensions of the inner margins of the chambers of the final whorl and by the fine secondary shell material. The 6 chambers of the final whorl are broader at the outer margins and pointed at the inner. On the dorsal side, they are as broad as high, non-inflated and less rapidly increasing in size whereas on the ventral side, they are broader than high, inflated and more rapidly increasing in size. On the dorsal side, the sutures are less distinct, arcuate, and flush with the surface but on the ventral side, they are more distinct, straight and depressed. The periphery is angled. The thin calcareous wall is smooth and somewhat coarsely perforate. The arch-shaped aperture with a thin lip is peripheral occurring at the base of the suboval apertural face and extends on the ventral side and typically on the dorsal side between the inner margins of the chambers and earlier whorls.

Remarks: A few specimens found in my samples are similar to *C. sandidgei* Brotzen.

ACKNOWLEDGEMENTS

I am grateful to the Vice-Chancellor of the Madras University for providing me the opportunity and facilities to conduct research. My thanks are due to the Geological Survey of India for having given me facilities in their magnificient Library at Calcutta.

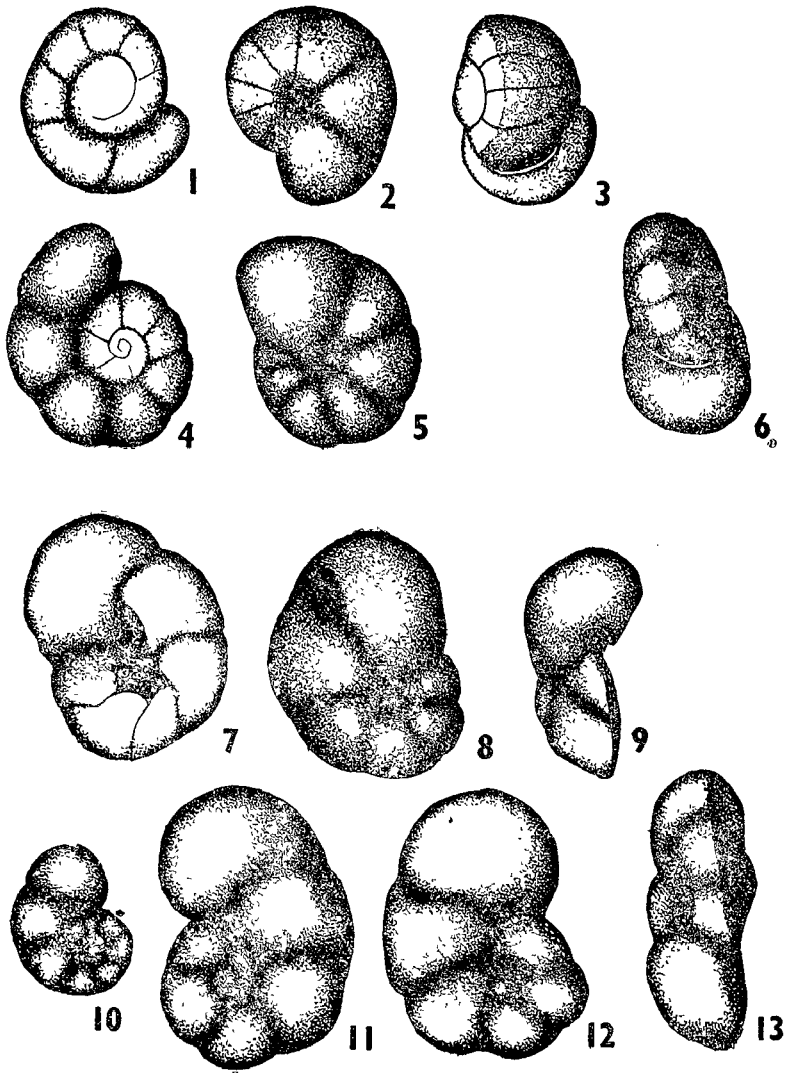

PLATE I

1-3 *Gyroidina globosa* (Hagenow) × 62.5. FIG. 1. Dorsal side; FIG. 2. Ventral side; FIG. 3. Peripheral view. 4-6 *Gyrodina loetterlei* Tappan. × 62 5. FIG. 4. Dorsal side; FIG. 5 Ventral side; FIG. 6. Peripheral view. 7-9 *Cibicides sandidgei* Brotzen × 62 5. FIG. 7. Dorsal side; FIG. 8. Ventral side; FIG. 9. Peripheral view. 10-13 *Discorbis minima* Vieaux × 62.5. FIG. 10. Dorsal side of a small specimen; FIG. 11. Dorsal side; FIG. 12. Ventral side. FIG. 13. Peripheral view.

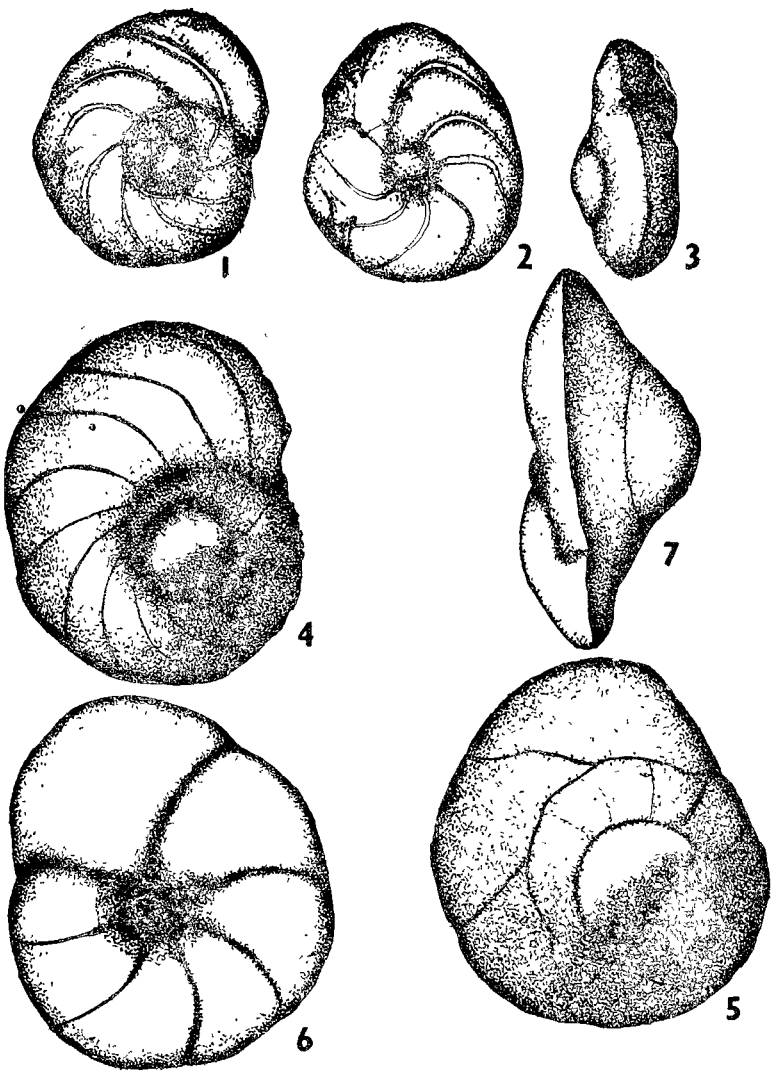

PLATE II

1-4 *Rotalia umbonella* Reuss. × 62 5. Fig. 1. Dorsal side; Fig. 2. Ventral side; Fig. 3. Peripheral view; Fig. 4. Dorsal side of a large specimen. 5-7 *Rotalia cullygoodiensis* sp. nov. × 62 5. Fig. 5 Dorsal side; Fig. 6. Ventral side; Fig. 7. Peripheral view

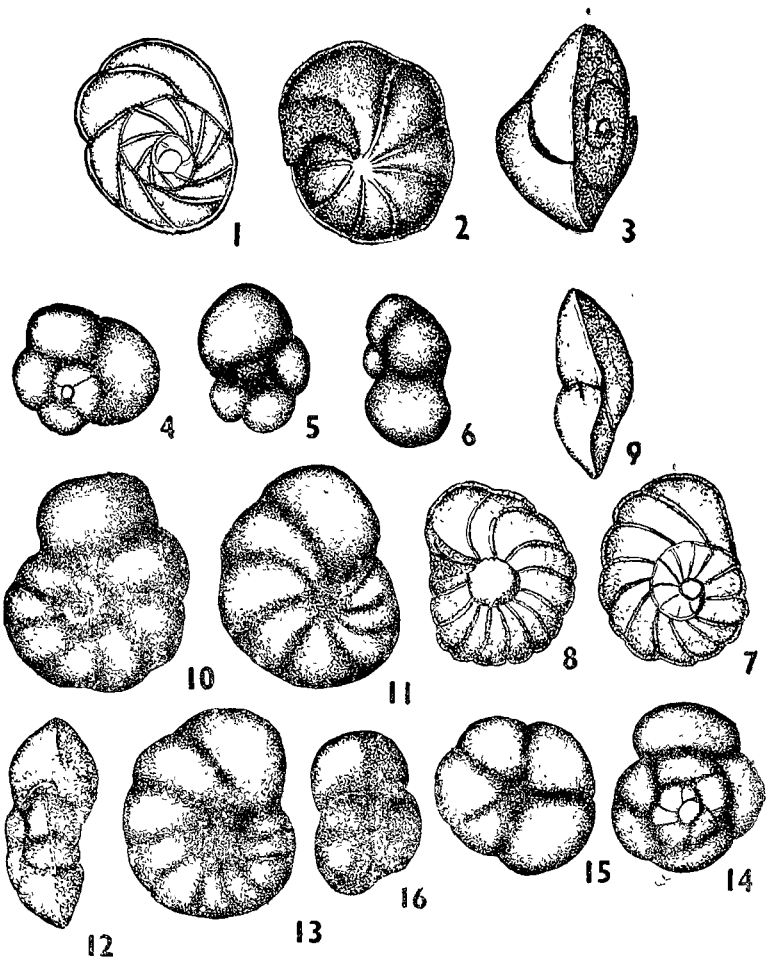

PLATE III

1-3 *Globorotalites michelinıana* d' Orbigny, × 62.5. FIG. 1. Dorsal side; FIG. 2. Ventral side; FIG. 3. Peripheral view. 4-6 *Globıgerina cretacea* var. *plana* Schacko × 100 FIG. 4 Dorsal side; FIG. 5. Ventral side; FIG. 6 Peripheral view. 7-9 *Parrella navarroana* (Cushman) × 62.5. FIG. 7 Dorsal side; FIG. 8. Ventral side; FIG. 9. Peripheral view. 10-13 *Anomalina intermedıa* Berthelin FIG 10 Dorsal side; FIG. 11. Ventral side; FIG. 12. Peripheral view; × 40 FIG. 13. Dorsal side of a specimen with depressed whorls × 62 5 14-16 *Discorbıs* sp × 100 FIG. 14. Dorsal side; FIG 15. Ventral side; FIG. 16. Peripheral view.

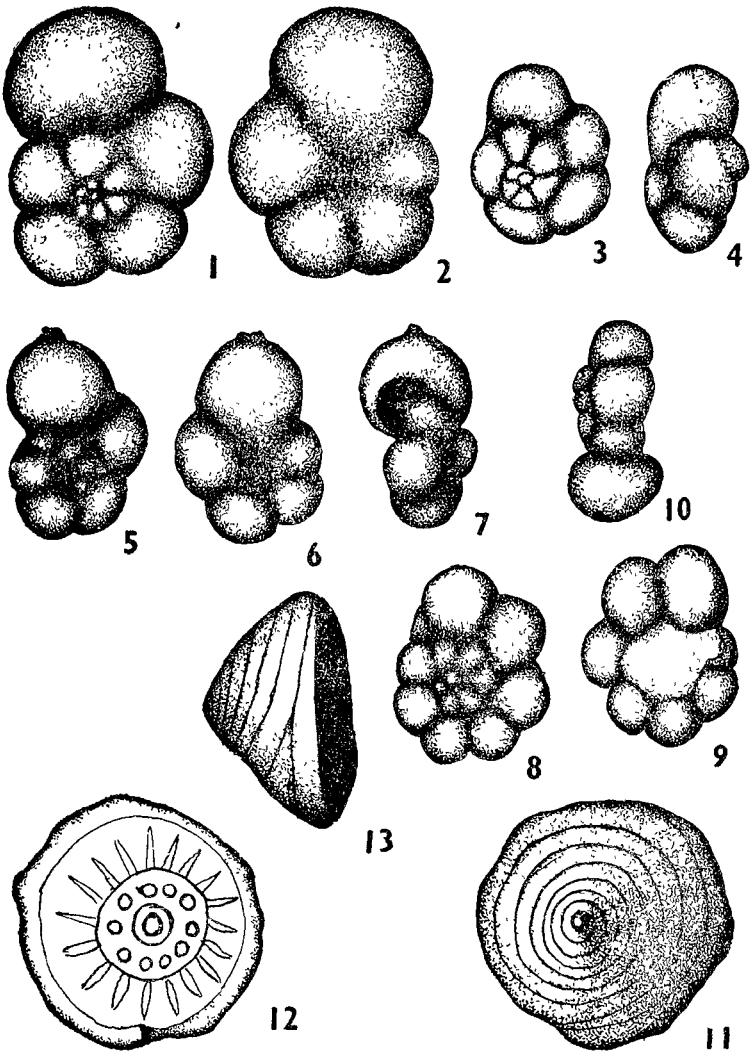

PLATE IV

1-4 *Globigerina cretacea* d' Orbigny FIG. 1. Dorsal side of a large specimen. FIG. 2. Ventral side of a large specimen. × 62.5 FIG. 3. Dorsal side of a small specimen; FIG. 4. Peripheral view. × 100. 5-7 *Globigerina* sp × 100. FIG. 5. Dorsal side; FIG. 6. Ventral side; FIG. 7. Peripheral view. 8-10 *Globigerina planispira* Tappan × 100. FIG. 8. Dorsal side, FIG. 9. Ventral side; FIG. 10. Peripheral view. 11-13. *Trocholin raoi* sp. nov. × 62 5 FIG 11. Dorsal side; FIG. 12. Ventral side; FIG. 13. Peripheral view.

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Anthracnose of Banana

I. STUDIES ON THE DISEASE

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The banana plant is subject to a large number of diseases wherever it is cultivated. One of the important diseases affecting the fruit is the anthracnose disease caused by *Gloeosporium musarum* Cke. and Mass. [*Colletotrichum musae* (Berk. & Curt.) v. Arx]. This disease is found wherever banana is grown, the fruit being liable to infection in the field, in storage and in transit.

The fungus causing the anthracnose disease was described by Cooke and Massee in 1873 from Brisbane. Cobb (1906) described the symptoms of the disease. Since then a great deal of investigation has been carried out by workers all over the world on this important disease.

The disease has generally been considered more as a storage and transit problem. Most of the studies so far had, therefore as their aim, the reduction of rotting and loss during storage. A few workers have however, recognised the importance of the disease as a field problem (Dastur 1916; Agati 1922; 1925; Serrano 1925; Toro 1925; Chona 1933; Jain 1950; Roy and Sharma 1952).

The disease has been known to occur in Madras State for a long time, but its importance has been recognised only in recent years when surveys showed that 10-15% of the bunches in the field were affected by this disease, most of the important commercial varieties being susceptible.

As already indicated most of the investigations on this disease have been on stored fruits. It is no doubt true that the results of some of these studies may be applicable under field conditions also. But there are still several aspects of the field disease which need investigation *de novo*. In the present studies an attempt has therefore, been made to fill these gaps in our knowledge regarding

this important disease. Since this disease was fairly widespread and occurred on many varieties of banana, it was considered worthwhile to investigate the variation in symptomatology and morphology of the pathogen on the host. The following aspects of the problem have therefore been investigated with special reference to variation.

1. Distribution of the disease in Madras State and varieties affected;
2. Symptomatology on the fruit and other parts of the plant;
3. Morphology of the fungus on the fruit on first collection;
4. Relationship of morphological and chemical characteristics of fruit to susceptibility;
5. Relationship of age of fruit to infection;
6. Varietal pathogenicity of the isolates.

MATERIALS AND METHODS

Isolation of the pathogen and its culture and maintainence were done according to the usual mycological techniques. The cultures were maintained on 4% oats agar slants.

Pathogenicity:

1. *Inoculation on host in the laboratory*: For cross inoculation studies, inoculations were made on mature hard bananas with spore suspension in sterile water. The density of suspension was adjusted in such a way that the spore density was approximately equal in all inoculations. The fruits were inoculated at two ends (inside a uniform circle made by a glass marking pencil) to locate the place of inoculation with drops of inoculum from a pipette of unifrom bore. Humidity was maintained by spraying sterile water once a day.

2. *Inoculation on the host in the field*: Inoculation on the bunch in the field was made by spraying spore suspension from an atomiser. The inoculated bunches were covered by alkathene bags for 72 hours to provide a humid atmosphere to initiate infection.

3. *Infection in relation to sugar content and thickness of peel*: For analysing the sucrose content of the fruits, the method mentioned in "Methods of analysis of Association of official Agricultural Chemists (1945)" was followed. The thickness of the peel was measured with the aid of a vernier calliper.

4. *Infection in relation to the age of the fruit*: This was studied by inoculating fruits from flower stage up to ripening stage in the laboratory by removing fingers from each hand which showed two days difference in age between hands.

Morphological:

The spores of the fungus from naturally infected hosts were measured. The length and breadth of one hundred such spores were measured in each isolate. Germination of spores were made in double distilled water. Drawings were made with the help of an Abbe Camera lucida.

EXPERIMENTAL

A survey of five districts of the State was made for assessing the incidence of the disease. The disease was observed on the varieties *Peyan* (Coimbatore and Tirunelveli), *Peykunnan, Kuribontha, Kaali* and *Pachanadan* (Coimbatore), *Musa balbisiana* (Coimbatore and Tanjore), *Rasthali* and *Malavazhai* (S. Arcot), *Peyladen, Karivazhai, Poovan, Walha, Krishnavazhai* and *Nendrapadathi* (Tanjore), *Monthan, Vayilvazhai, Ayiramkarasthali, Virupakshi, Aattukomban* and *Neyvazhai* (Madurai) and *Padathi* and *Kadali* (Tirunelveli) and *Vellavazhai* (Coimbatore and Madurai). About 10-15% of the bunches were affected in all the areas surveyed.

Symptomatology on fruits and other parts of plants:

The symptoms were found to vary with varieties. In most of the cases as in *Poovan, Musa balbisiana* (Kallar) and *Musa balbisiana* (Aduthurai), *Krishnavazhai* and *Vayilvazhai* immature fruits were affected. The effect of the disease in these cases was, premature ripening of the fruit. The initial anthracnose lesion enlarged rapidly involving the entire fruit in a soft rot. On the rotted fruit acervuli were produced profusely.

Another symptom of common occurrence was the browning or the blackening of mature and immature fruits without any lesions with profuse orange acervuli as in varieties *Rasthali, Karivazhai, Walha, Nendrapadathi, Peyan* (Coimbatore), *Pachanadan, Kadali, Ayiramkarasthali, Virupakshi* and *Neyvazhai*.

In the varieties *Kaali* and *Peyladan*, the symptom exhibited was different from the above in not having the blackened area. Instead, watersoaked oily lesions were produced on which acervuli were formed.

On the rachis and green fruits of *Malavazhai* and *Kuribontha*, the presence of the disease was indicated by small circular specks on the skin which increased in size, splitting the rachis.

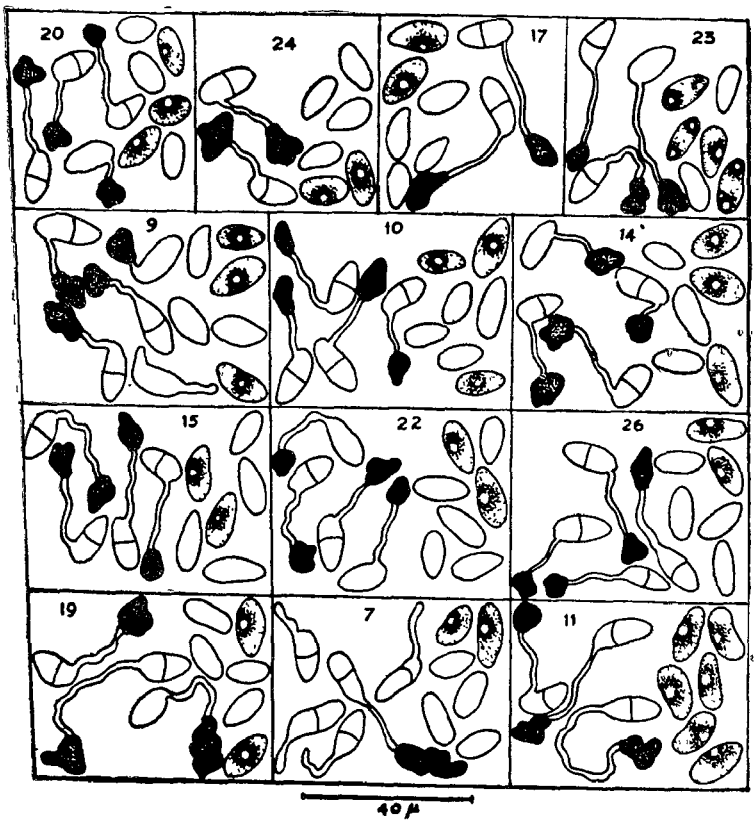

FIG. 1. Conidial size, shape and measurements.

Isolate Numbers: Group 1. 20 from Monthan; 24 from Neyvazhai; Group 2. 17 from Peyan (Tirunelveli); 23 from Vellavazhai (Pannakad). 9 from Musa balbisiana (Aduthurai); 10 from Musa balbisiana (Kallar); 14 from Krishnavazhai; 15 from Vellavazhai (Coimbatore); 16 from Kadali; 17 from Vayilvazhai; 18 from Ayiramkarasthali; 19 from Peyan (Coimbatore); 20 from Kuribontha.

This advanced towards the hand, then to the pedicel and finally to the fingers. In the advanced stages of the disease, the specks on the fruits of *Kuribontha* became sunken merging into one another forming large spots, thus involving the entire fruit, turning the colour to black over which were found pink spore beds.

Another common feature of the symptom exhibited by the variety *Peykunnan* and *Monthan* was, that as soon as the fruits reached a length of 3 inches, they began to change colour and to shrivel, passing through greenish yellow, yellow, brown to almost black appearing like sun scald accompanied by pink eruptions of *Gloeosporium* acervuli. There were no distinct lesions. The fruits became shrivelled in a short time.

In some cases as in *Vellavazhai* (Coimbatore), *Peyan* (Tirunelveli), *Padathi, Vellavazhai* (Pannakad) and *Aattukomban*, the diseased fruits turned black from the point of attack and the whole

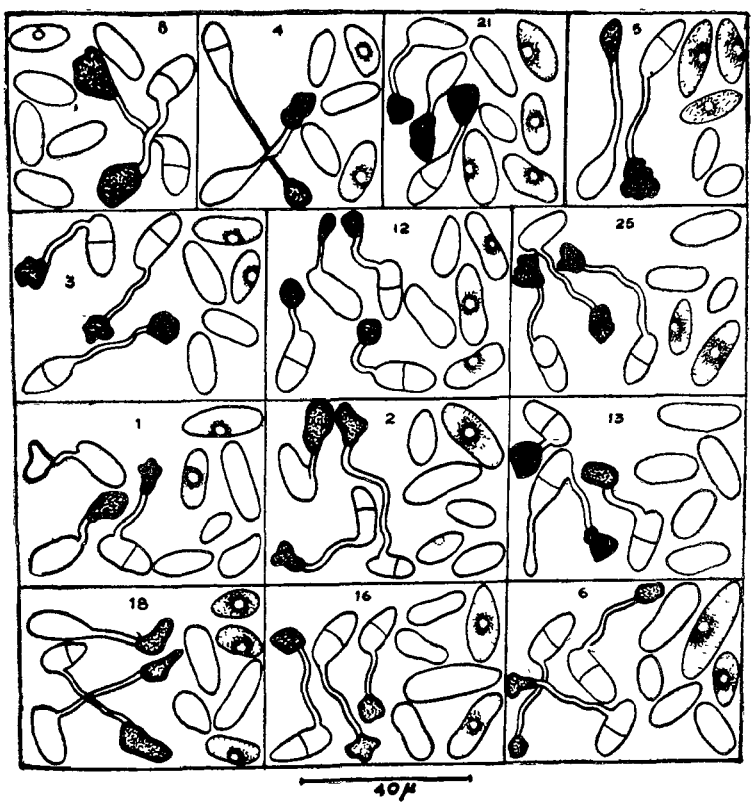

FIG. 2. Conidial size, shape and measurements.

Isolate Numbers: Group 3. 8 Walha; 4 Nendrapadathi; Group 4. 21 Virupakshi; 5 Poovan; 3. Karivazhai; 12 Peykunnan; 24 Attukomban; Group 5. 1 Kaali; 2 Peyladen; 13 Rasthali; 18 Padathi; Group 6. 16 Pachanadan, 6 Malavazhai.

TABLE I

Giving the description of conidia and conidial measurements on the fruits

Groupings based on length of the conidia	Description of the conidia	Isolates coming under the group	Spore measurements
1. Maximum length not exceeding 15 μ	Conidia oval, rounded at one end and tapering towards the other. Some conidia cylindrical with parallel sides and rounded ends with one oil globule in the centre.	*Monthan*	9·8 — 14 × 4·2 — 6·7 μ (12·6 × 5·2) μ
		Neyvazhai	9·8 — 14·4 × 4·9 — 7·0 μ (11·2 × 5·2) μ
2. Maximum length not exceeding 17 μ	Conidia oval, blunt at one end and tapering at the other with one oil globule in the centre.	*Peyan* (Tirunelveli)	8·4 — 15·4 × 5·2 — 7·0 μ (12·6 × 5·6) μ
		Vellavazhai (Pannakad)	10·5 — 15·4 × 4·2—6·3 μ (12·6 × 5·2) μ
	In *Vellavazhai* (Pannakad). the conidia are mostly with two oil globules.	*Musa balbisiana* (Aduthurai)	9·8—16·8 × 4·2 — 7·0 μ (11·2 × 5·6) μ
		Musa balbisiana (Kallar)	8·9 — 16·8 × 4·9 — 9·8 μ 12·6 × 5·2) μ
		Krishnavazhai	9·1 — 16·8 × 4·9 — 7·0 μ (14 × 5·6) μ
		Vellavazhai (Coimbatore)	8·4 — 16·8 × 4·9 — 7·7 μ 12·6 × 5·6) μ
		Kadali	9·1 — 16·8 × 4·9 — 6·3 μ (11·2 × 5·2) μ

	In *Vayilvazhai, Ayiramka rasthali* and *Peyan* (Coimbatore) isolates, the conidia are cylindrical.	*Vayilvazhai*	11·2 — 16·2 × 4·9 — 7·0 μ (14 × 5·6) μ
		Ayiramka-rasthali	9·8 — 15·4 × 4·2 — 6·3 μ (11·2 × 5·2) μ
		Peyan (Coimbatore)	9·8 — 15·4 × 4·9 — 7·0 μ (12·6 × 5·2) μ
	In *Kuribontha* isolate the conidia are bean shaped with both ends rounded.	*Kuribontha*	8·4 — 15·4 × 4·2 — 7·0 μ (12·6 × 5·2) μ
3. Maximum length not exceeding 19 μ	Cylindrical, hyaline blunt at one end and tapering at the other with one oil globule in the centre.	*Walha*	11·2 — 18·2 × 4·9 — 7·0 μ (14 × 5·2) μ
		Nendra-padathi	9·8 — 18·2 × 4·9 — 7·0 μ (14 × 5·2) μ
4. Maximum length not exceeding 21 μ	In *Virupakshi* and *Poovan* isolates, the conidia are oval, rounded at one end and tapering towards the other. Hyaline with one oil globule in the centre.	*Virupakshi*	9·8 — 19·6 × 5·6 — 7·7 μ (12·6 × 5·2) μ
		Poovan	7·0 — 21·0 × 4·9 — 6·3 μ (11·2 × 5·2) μ
	Conidia cylindrical hyaline, blunt at one end and tapering at the other with one oil globule in the centre. In the case of *Peykunnan* there is a constriction in the middle giving a dumbbell appearance.	*Karivazhai*	10·5 — 19·6 × 4·9 — 6·3 μ (12·6 × 5·2) μ
		Peykunnan	11·2 — 21 × 5·6 — 8 μ (14 × 7) μ
		Aattukomban	9·8 — 21 × 4·9 — 7·0 μ (12·6 × 5·6) μ

Groupings based on length of the conidia	Description of the conidia	Isolates coming under the group	Spore measurements
5. Maximum length not exceeding 23 μ	Cylindrical, hyaline blunt at one end and tapering at the other with a single oil globule in the centre. In *Rasthali* the oil globules are very rare.	*Kaali*	9·8 — 22·4 × 4·2 — 7·0 μ (12·6 × 5·2) μ
		Peyladen	11·2 — 22·4 × 5·6 — 7·0 μ (14 × 5·6) μ
		Rasthali	9·8 — 22·4 × 4·9 — 7·0 μ (12·6 × 5·2) μ
	In *Padathi* both oval and cylindrical spores are observed with a single oil globule.	*Padathi*	9·8 —22·4 × 4·2 — 9·1 μ (12·6 × 5·2) μ
6. Maximum length exceeding 23 μ	Mostly oval rounded at one end and tapering at the other. Some are cylindrical with a constriction in the middle which gives a dumb-bell shaped appearance with one oil globule in the centre.	*Pachanadan*	8·4 — 25·2 × 4·2 — 8·4 μ (14·4 × 5·6) μ
	Cylindrical, hyaline blunt at one end and the other tapering. Mostly with one oil globule in the centre.	*Mala vazhai*	9·8 — 29·4 × 4·9 — 7·0 μ (12·6 × 5·6) μ

TABLE II

Intensity of infection in relation to sugar content and thickness of peal using four isolates of *G. musarum* on 20 varieties of banana

Isolates of *G. musarum*	Vellavazhai Isolate			Peyan Isolate			Kaali Isolate			Kuribontha Isolate			Sugar Content		
	Infection	Diameter of lesions	Incubation period	Infection	Diameter of lesions	Incubation period	Infection	Diameter of lesions	Incubation period	Infection	Diameter of lesions	Incubation period	Reducing sugar	Sucrose	Thickness of peel in cms
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
Varieties of Banana															
1. Chenkadali	+	5 mm	12 days	+	7 mm	12 days	+	4 mm	12 days	+	9 mm	12 days	5%	11%	0·26 cm
2. Thellabontha	+	9 mm	18 days	+	15 mm	18 days	+	3 mm	18 days	+	23 mm	18 days	16·6%	0·85%	0·18 cm
3. Vellavazhai	+	19 mm	12 days	+	5 mm	10 days	+	5 mm	10 days	+	20 mm	10 days	18·51%	1·8%	0·14 cm
4 Aattunendran	+	5 mm	13 days	+	7 mm	13 days	+	13 mm	13 days	+	13 mm	13 days	7·8%	9·24%	0·15 cm
5. Vamanakeli	+	10 mm	5 days	+	20 mm	5 days	+	12 mm	5 days	+	30 mm	5 days	10%	6·6%	0·15 cm
6. Peykunnan	+	5 mm	16 days	+	18 mm	16 days	+	10 mm	16 days	+	18 mm	16 days	14·28%	9·2%	0·17 cm
7. Peyan	+	13 mm	14 days	+	25 mm	14 days	+	20 mm	14 days	+	30 mm	14 days	14·28%	3·2%	0·23 cm
8. Kullan	—	—	—	—	—	—	—	—	—	+	5 mm	12 days	10·64%	10·09%	0·15 cm
9 Pachanadan	—	—	—	+	8 mm	8 days	+	3 mm	8 days	+	10 mm	8 days	11·63%	10·04%	0·22 cm
10. Neyvannan	—	—	—	+	10 mm	12 days	+	12 mm	12 days	+	10 mm	12 days	8·47%	6·8%	0·18 cm
11. Boodithamontha bathees	+	10 mm	12 days	+	20 mm	12 days	+	15 mm	12 days	+	25 mm	12 days	8·47%	3·25%	0·24 cm
12. Rajavazhai	+	12 mm	16 days	+	15 mm	16 days	+	12 mm	16 days	+	20 mm	16 days	11·63%	4·28%	0·195 cm
13. Nendrapadathi	+	8 mm	7 days	+	10 mm	7 days	+	10 mm	7 days	+	15 mm	7 days	6·56%	11·27%	0·21 cm
14. Poovan	—	—	—	+	15 mm	7 days	—	—	—	+	25 mm	7 days	16·66%	4·24%	0·18 cm
15. Malavazhai	+	25 mm	8 days	+	10 mm	8 days	+	20 mm	8 days	+	30 mm	8 days	13·51%	9·87%	0·16 cm
16. Virupakshi	+	5 mm	7 days	+	10 mm	7 days	—	—	—	+	20 mm	7 days	12 8%	8·93%	0·23 cm
17. Krishnavazhai	—	—	—	+	12 mm	5 days	+	10 mm	5 days	+	20 mm	5 days	13·6%	10·26%	0·21 cm
18. Neypoovan	—	—	—	—	—	—	+	10 mm	8 days	+	15 mm	8 days	7·46%	8·24%	0·14 cm
19. Kaali	—	—	—	+	10 mm	5 days	+	40 mm	5 days	+	30 mm	5 days	15·15%	2·59%	0·15 cm
20. Monthan	—	—	—	—	—	—	+	15 mm	17 days	+	20 mm	17 days	13·51%	2·99%	0·24 cm

fruit was involved in a short time. The diseased fruits finally shrivelled and dried but remained attached to the central stalk and the acervuli were not formed unless the fruits were incubated in a moist chamber.

Morphology of the fungus on the fruit on first collection:

To study the morphology of the conidia, drawings were made with oil immersion objective with a camera lucida (Figs. 1 and 2). The spores used were from acervuli produced on affected fruits on first collection. The descriptions and measurements of spores of isolates of *G. musarum* affecting 26 varieties are presented in Table I.

Even though the maximum length of the conidia showed a variation from 14 to 29 μ, the mean lengths varied only within a narrow range of 9.8 to 14.4 μ.

Relationship of morphological and chemical characters of fruit to susceptibility:

Research in Jamaica, Philippines and Puerto Rico has indicated that sugar content of the fruit as well as thickness of peel have important roles in determining susceptibility and that sweeter varieties are more susceptible to the disease. To test this, mature hard bananas of both the sweet table varieties and insipid cooking varieties were inoculated with four isolates of *G. musarum* namely the isolates from *Kaali, Peyan, Kuribontha* and *Vellavazhai* Similar fruits were kept as control. The control fruits were analysed for both reducing sugars and sucrose content following A.O.A.C. (1945) methods.

The thickness of the peel of banana was ascertained with the use of vernier callipers and is expressed in centimeters.

Details of the intensity of infection in relation to sucrose content and thickness of peel are given in Table II.

The reducing sugar content of the fruit is found to be positively associated with diameter of lesions and the incubation period (Table IIa).

This would mean that increased reducing sugar content while accelerating the fungal growth, relatively retards the formation of fructification. On the other hand sucrose content is negatively correlated with diameter of lesions and incubation period, indicating

TABLE II(a)

Measurement of association between reducing sugars, sucrose and thickness of peel of 20 banana varieties and the intensity of infection and activity of isolates of *G. musarum* as measured by the diameter of lesions and incubation period respectively.

Kinds of association.	Sl. No.	Between	Value of 'r' or 'b'	Level of significance	Remarks
Total Correlations	1	Reducing sugars in fruit and diameter of lesions.	0 489	**	
	2	Reducing sugars in fruit and incubation period	0.403	**	
	3	Sucrose and diameter of lesion.	0.324	**	
	4	Sucrose and incubation period.	0.362	**	
	5	Thickness of peel and diameter of lesion.	0 124	—	
	6	Thickness of peel and incubation period.	0.187	—	
	7	Reducing sugars and sucrose content.	0.59	**	
	8	Diameter of lesion and incubation period.	0·137	**	
Partial Correlation	1	Reducing sugars and diameter of lesion eliminating No 3 above.	0·378	*	
	2	Reducing sugars and incubation period eliminating No. 4 above.	0.252	*	
	3	Sucrose and diameter of lesion eliminating No. 1 above.	0.076	—	
	4	Sucrose and incubation period eliminating No. 2 above.	0·168	—	
Regressions	1	Regression of diameter of lesion Y on reducing sugars X.	0·802	*	Y=5 31 + 0 802.X
	2	Regression of incubation period Y on reducing sugar X.	0·366	**	Y=6 66 + 0 366 X
	3	Regression of diameter of lesion Y on the sucrose content X	0·744	**	Y=19 28 + 0 744.X
	4	Regression of incubation period Y on sucrose content X.	0.43	**	Y=13.63 + 0 43 X

** Significant at P = 0.01 level

* Significant at P = 0·05 level.

exactly the opposite effect of reducing sugars i.e., sucrose content retards growth but accelerates formation of fructification. Both these results are sustained indirectly by the fact that there is a strong negative correlation between reducing sugars and sucrose content in the fruit. The two relationship viz., reducing sugars and diameter of lesion and incubation period on the one hand and sucrose and the two features on the other, would thus appear to mean one and the same thing.

It also follows that on any given fruit, the diameter of lesions and incubation period are characteristic for a given isolate.

The relationship between (1) reducing sugars and diameter of lesions and incubation period and (2) sucrose content and diameter and incubation period is evaluated by the four regression coefficients all of which are highly significant. These coefficients measure the rate of change in the diameter and incubation period for unit change in reducing sugars and sucrose content.

The two partial correlations between reducing sugars on the one hand and diameter of lesions on the other after eliminating the influence of sucrose, are both significant but are of less magnitude than when the influence of sucrose is not eliminated. This shows that sucrose too has some effect on these two features and that the influence of reducing sugars on them is not exclusive . However, when the influence of reducing sugars is eliminated sucrose is found to have no correlation with the diameter and incubation period as shown by the two non-significant negative values. It is therefore, to be concluded that the reducing sugar content is by far the more important in its influence on the two features.

Relationship of age of the fruit to infection:

Infection in relation to age of the fruit was studied by inoculating fruits immediately after flower opening, up to ripening stage in the laboratory by removing fingers from each hands which showed a difference of 2 days in age between hands.

Four isolates of *G. musarum* from *Kaali, Peyan, Kuribontha* and *Vellavazhai* (Coimbatore) were inoculated on fruits of their respective hosts to ascertain at what stage the fruits get infected. For this purpose the fruits were inoculated both in the field and the laboratory on detached fruits (Fig. 3 and 4).

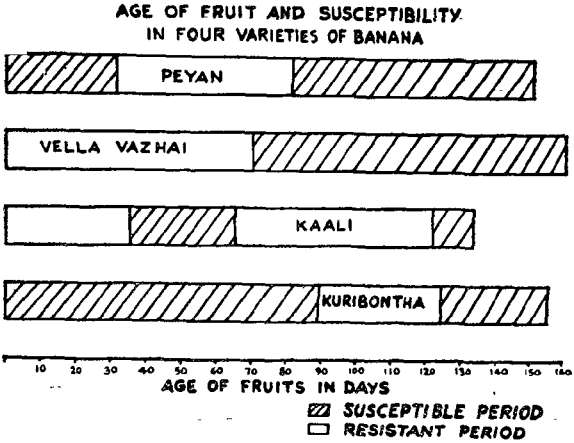

FIG. 3. Age of fruit and susceptibility in four varieties of banana.

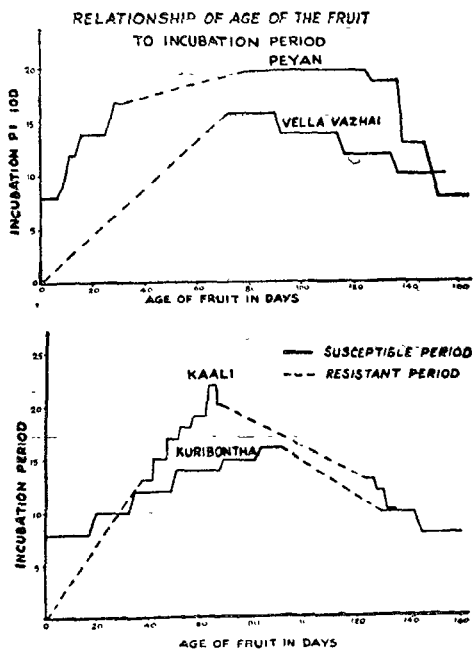

FIG. 4. Relationship of age of the fruit to incubation period.

The results of laboratory inoculation showed that in *Peyan* the fruits were susceptible from flowers stage to 30th day. From 31st day to 78th day, the fruits were not susceptible. From

80th day to 150th day (ripening stage) the fruits were again susceptible.

In the case of *Vellavazhai* the fruits got infected from 70th to 160 days (ripening stage). They were not infected up to 68 days.

In the case of *Kaali* infection was not noticed from flower stage to 35 days old fruits. Infection started from 36th day and continued up to 65 days old fruits. There was no infection of fruits of maturity ranging from 66 days to 122 days. The fruits again took infection from 125 to 134 days (ripening stage).

As regards *Kuribontha* the fruits got infected from flower stage up to 90 days. From 91st day to 126th day there was no infection. But the fruits continued to be susceptible thereafter up to 156th day (ripening stage) (Table III).

TABLE III

Showing infection in relation to age of the fruit of four varieties of banana.

Peyan		Vellavázhai	
Age of fruits	Incubation period	Age of fruits	Incubation period
2- 6 days	9 days	1- 68 days	No infection
8 days	10 days	70- 88 days	16 days
10- 12 days	12 days	90-112 days	14 days
14- 24 days	24 days	114-132 days	12 days
26- 30 days	17 days	134-146 days	10 days
32- 78 days	No infection	148-160 days	8 days
80-122 days	20 days		
124-132 days	19 days		
134-142 days	13 days		
144-150 days	10 days		

Kaali		Kuribontha	
Age of fruits	Incubation period	Age of fruits	Incubation period
1- 35 days	No infection	2- 18 days	8 days
36- 40 days	13 days	20- 32 days	10 days
41- 45 days	15 days	34- 48 days	12 days
46- 50 days	17 days	50- 66 days	14 days
51- 55 days	18 days	68- 80 days	15 days
56- 60 days	19 days	82- 90 days	16 days
61- 63 days	22 days	92-126 days	no infection
64- 65 days	20 days	128-140 days	10 days
66-122 days	no infection	142-156 days	8 days
123-125 days	13 days		
126-130 days	12 days		
131-134 days	10 days		

Two varieties *Peyan* and *Kuribontha* agreed in having an initial susceptible period, a middle non susceptible period and a final susceptible period. The actual ages at which the fruits were not susceptible, however, differed. *Peyan* fruits were not susceptible from 32nd to 78th day, while *Kuribontha* fruits were non-susceptible from the 92nd to 126th day.

The varieties *Vellavazhai* and *Kaali* differed from *Peyan* and *Kuribontha* and also between each other in their behaviour. *Vellavazhai* had only one non-susceptible period i.e., from fruitset to 68th day while in *Kaali* there were two such periods i.e., from fruitset to 35th day and from 66th day to 122nd day.

In all the varieties, the incubation period showed a characteristic fluctuation. In the *Peyan* variety, the fruit remained susceptible up to 30 days from fruitset. But the incubation period rose from 9 days when inoculation was done on date of fruitset, to 17 days when inoculation was done on 30 day old fruits. Thereafter the fruits remained non susceptible when inoculation was done on the 32-78 day. The fruits again became susceptible when inoculation was done on the 80th day, the incubation period being 20 days. This remained steady till the 122nd day. Thereafter there was a continuous fall, till the fruits started to ripen when the incubation period was 10 days.

In the *Vellavazhai* variety, the fruits were non-susceptible up to 68 days from fruitset. Thereafter the fruits remained susceptible, when the inoculation was done on 70-160 days old fruits (ripening stage). The incubation period remained steady at 16 days when fruits up to 88 days were inoculated. Thereafter up to 112 days, the incubation period was 14 days. The incubation period was twelve days when inoculations were done from 114-132 days, 10 days from 134-146 days and 8 days from 148-160 days.

As regards *Kaali* variety, the fruits were non-susceptible up to 35 days from fruitset. The fruits were susceptible thereafter when inoculation was done on 36 to 65 days old fruits. The incubation period rose from 13 days when inoculation was done on 36 days old fruits, to 22 days when inoculation was done on 65 days old fruits. The fruits again remained non-susceptible when inoculation was done on 66-122 days old fruits. The fruits again became susceptible. When inoculation was done on 123 days old fruits, the incubation period was 13 days and there was a fall in the incubation period to 12 days and when the fruits started ripening, incubation period was 10 days.

In the variety *Kuribontha*, the fruits remained susceptible up to 90 days from fruit set. The incubation period rose from 8 days when inoculation was done on date of fruit set, to 16 days when inoculation was done on 90 days old fruits. Thereafter the fruits remained nonsusceptible for a short period when inoculated on 92 to 126 days old fruits. The fruits again became susceptible. When inoculation was done on 128 to 140 days old fruits, the incubation period was 10 days. Thereafter the incubation period fell to 8 days up to 156 days old fruits (ripening stage).

The inoculation in the field on the four varieties with their respective isolates showed a similar behaviour to what was observed in the laboratory (Plate Figures I, II, III and IV).

Pathogenicity of 26 isolates of G. musarum on 31 varieties of banana:

The twenty six isolates of *G. musarum* were inoculated on 31 varieties of banana available at Coimbatore to test their pathogenicity. The varieties were *Kaali, Padathi, Neyvazhai, Kullan, Krishnavazhai, Nendrapadathi* and *Malavazhai* coming under

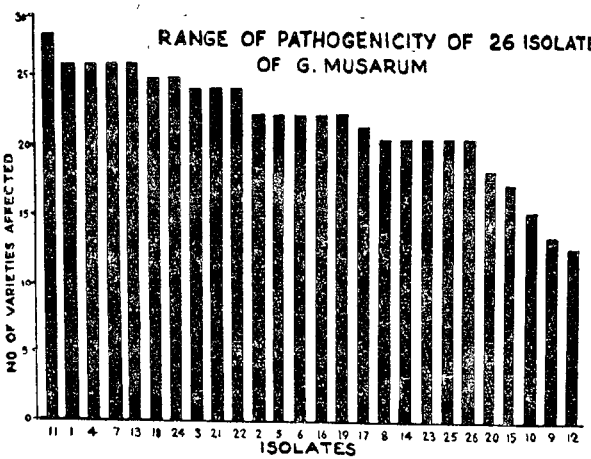

FIG. 5. Range of pathogenicity of twenty-six isolates of *G. musarum*.

Kaali or *Nadan* group.* *Thellabontha Kuribontha, Monthan, Boodıtha montha bathees* coming under *Monthan* group. *Vayilvazhai, Peyladen, Peyan, Peykunnan, Rajavazhai* coming under *Peyan* group. *Kunnan, Thattillakunnan, Neypoovan* under *Kunnan* group. *Poovan Rasthali* under *Poovan* group, *Nendran*

* The groups are designated according to Jacob (1952)

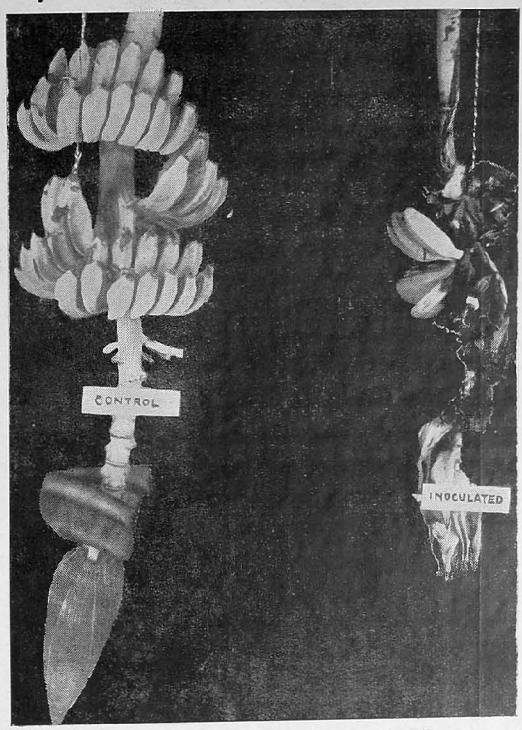

FIG. I

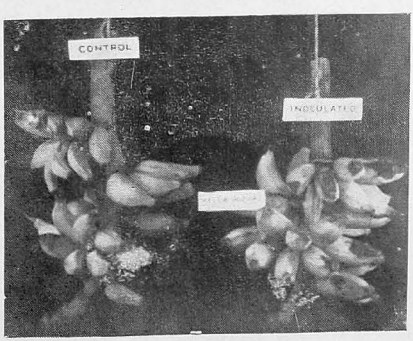

FIG. II

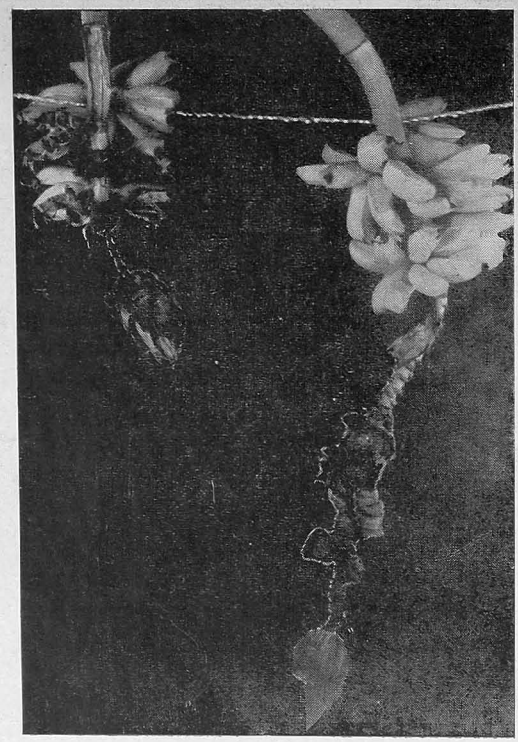

FIG. III

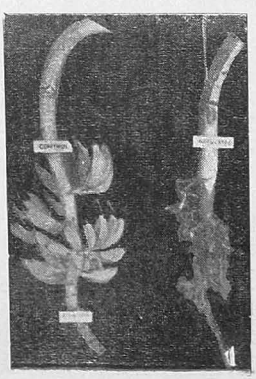

FIG. IV

under *Nendran* group, *Kadali, Karivazhai, Aattukomban* coming under *Kadali* group, *Vellavazhai, Pachanadan, Ayiramkarashali, Vayilvazhai* and *Musa balbisiana* and *Musa chiliocarpa* (wild bananas).

There was great variation in the range of pathogenicity of the isolates as shown in Table IV and Fig. 5. This experiment had shown that the isolate from *Kuribontha* was the most virulent affecting the maximum number of varieties namely 29. Next in order were the isolates from *Kaali, Nendrapadathi, Peyan* (Coimbatore) and *Rasthali. Peykunnan* isolate affected the least number being thirteen. All the rest of the isolates infected varieties in between these extremes.

TABLE IV

Showing range of pathogenicity of 26 isolates of *G. musarum* on 31 varieties of banana

Gloeosporium isolates from	Number of varieties infected	% of varieties infected
Kuribontha	29 varieties	93·5
Kaali	27 ,,	87·1
Nendrapadathi	27 ,,	87·1
Peyan (Coimbatore)	27 ,,	87·1
Rasthali	27 ,,	87·1
Padathi	26 ,,	83·9
Neyvazhai	26 ,,	83·9
Virupakshi	26 ,,	83·9
Kadali	26 ,,	83·9
Karivazhai	25 ,,	80·6
Peyladen	23 ,,	74·2
Poovan	23 ,,	74·2
Malavazhai	23 ,,	74·2
Ayiramkarasthali	23 ,,	74·2
Pachanadan	23 ,,	74·2
Peyan (Tirunelveli)	22 ,,	70·9
Vellavazhai (Pannakad)	21 ,,	67·7
Aattukomban	21 ,,	67·7
Vayilvazhai	21 ,,	67·7
Walha	21 ,,	67·7
Krishnavazhai	21 ,,	67·7
Monthan	20 ,,	64·5
Vellavazhai (Coimbatore)	17 ,,	54·8
Musa balbisiana (Kallar)	16 ,,	51·6
Musa balbisiana (Aduthurai)	14 ,,	45·1
Peykunnan	13 ,,	41·9

TABLE V

Showing percentage of isolates of *G. musarum* affecting each variety

Varieties of banana	Number of isolates infecting	% of isolates infecting	Sweetness or otherwise of the variety
Malavazhai	26	100%	sweet
Kunnan (AB)	26	100%	sweet
Thellabontha (ABB)	26	100%	insipid
Musa chiliocarpa (AA)	26	100%	insipid
Nendrapadathi (AAB)	25	96·1	sweet
Musa balbisiana (B)	24	92·3	insipid
Virupakshi (AAB)	24	92·3	sweet
Peykunnan (ABB)	22	84·6	sweet
Nendran (AAB)	21	80·8	sweet
Thattillakunnan (ABB)	21	80·8	sweet
Karivazhai (AAA)	20	76·9	sweet
Poovan (AAB)	20	76·9	sweet
Kuribontha (ABB)	20	76·9	insipid
Pachanadan (AAB)	20	76·9	sweet
Krishnavazhai (AAB)	19	73·1	sweet
Vellavazhai	19	73·1	sweet
Neypoovan (AB)	19	73·1	sweet
Padathi	18	69·2	insipid
Kullan (AAB)	18	69·2	sweet
Rajavazhai (AAA)	18	69·2	sweet
Kaali (AAB)	17	65·4	sweet
Aattukomban	17	65·4	sweet
Peyladen (ABB)	16	61·5	sweet
Rasthali (AAB)	16	61·5	sweet
Ayiramkarasthali (AAB)	16	61·5	sweet
Vayilvazhai	16	61·5	sweet
Monthan (ABB)	15	57·7	insipid
Kadali (AA)	14	53·8	sweet
Neyvazhai	12	46·2	sweet
Peyan (ABB)	10	38·5	sweet
Booditha montha bathees (ABB)	4	15·4	insipid

There was also great variation in the susceptibility of the varieties to infection (Table V and Fig. 6), while the four varieties *Thellabontha, Kunnan, Musa chiliocarpa* and *Malavazhai* were infected by all the isolates, one variety *Booditha montha bathees* was infected by only four of the isolates. None were immune. All the rest of the varieties were in between these extremes.

It further showed that the intensity of infection in terms of incubation period, was not the same on all the varieties, nor was it equal with all the isolates. Variations were observed in the incubation period (Table VI).

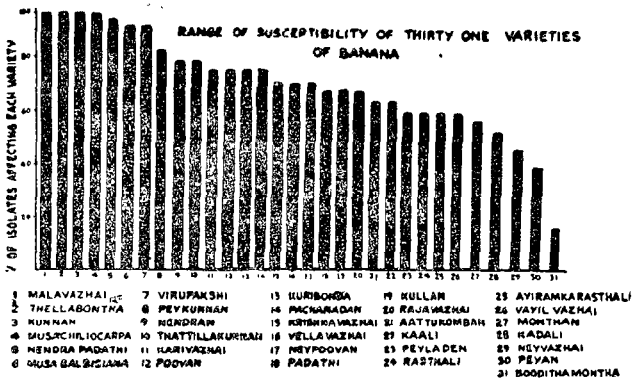

FIG. 6. Range of susceptibility of thirty-one varieties of banana.

TABLE VI

Showing intensity of infection in terms of incubation period of 26 isolates of *G. musarum* on 31 varieties

Varieties	Incubation period with 26 isolates
Nendrapadathi (AAB)	Infected within 5 to 7 days
Malavazhai (ABB)	,, ,, 5 to 7 days
Musa chilliocarpa (AA)	,, ,, 5 to 7 days
Kaali (AAB)	,, ,, 5 to 9 days
Peyan (ABB)	,, ,, 5 to 9 days
Kunnan (AB)..	,, ,, 5 to 9 days
Kadali (AA)	,, ,, 6 to 9 days
Karivazhai (AAA)	,, ,, 5 to 10 days
Thattillakunnan (ABB)	,, ,, 7 to 10 days
Padathi	,, ,, 8 to 10 days
Rasthali (AAB)	,, ,, 4 to 11 days
Booditha montha bathees (ABB)	,, ,, 12 days
Poovan (AAB)	,, ,, 4 to 12 days
Kullan AAB	,, ,, 4 to 12 days
Virupakshi (AAB)	,, ,, 4 to 12 days
Neypoovan (AB)	,, ,, 7 to 12 days
Krishnavazhai (AAB)	,, ,, 7 to 12 days
Peyladen (ABB)	,, ,, 9 to 12 days
Aattukomban	,, ,, 9 to 12 days

Varieties	Incubation period with 26 isolates
Vayilvazhai	9 to 12 days
Neyvazhai	10 to 12 days
Pachanadan (AAB)	4 to 13 days
Vellavazhai	5 to 15 days
Nendran (AAB)	6 to 15 days
Peykunnan (ABB)	15 to 16 days
Thellabontha	12 to 17 days
Kuribontha (ABB)	6 to 18 days
Monthan	13 to 18 days
Rajavazhai (AAB)	15 to 20 days
Ayiramkarasthali (AAB)	4 to 21 days
Musa balbisiana	10 to 25 days

The isolates of *G. musarum* from *Kaali, Peyladen, Karivazhai Nendrapadathi, Peyan* (Coimbatore), *Musa balbisiana* (Kallar) and *Krishnavazhai, Peyladen, Poovan, Malavazhai, Walha, Kuribontha, Rasthali, Pachanadan, Monthan, Virupakshi, Kadali, Vellavazhai, Neyvazhai, Aattukomban, Vayilvazhai* took 17-25 days to infect varieties like *Musa balbisiana, Rajavazhai* and *Ayiramkarasthali* and the same isolates took only 4-6 days on *Pachanadan, Poovan, Virupakshi, Nendrapadathi, Kunnan, Malavazhai, Kaali, Kullan, Musa chiliocarpa.*

It was noticed that in general, all the isolates required a long incubation period of 16 to 25 days on variety *Musa balbisiana* and 18-20 days on *Rajavazhai,* while all the isolates on *Nendrapadathi* took a minimum incubation period of 4-7 days, 5-9 days on *Kaali,* 5-7 days on *Malavazhai* and 5-9 days on *Kunnan.*

DISCUSSION

Anthracnose caused by *G. musarum* [*Collototrichum musae* (Berk. and Curt.) v. Arx] is of common occurrence wherever bananas are grown. The disease as occurring in the plantation, curing pits, storage and markets has been described by Agati (1922) from Philippine islands, Ashby, (1922) from Jamaica Laubert (1926) from Germany, Simmonds (1928) and Toro (1922) in Puerto Rico, Park (1930; 1933) in Ceylon, Wardlaw (1930; 1931; 1940) in Trinidad and Haiti, Leach (1959) in Jamaica. The comprehensive work of Tomkins (1931) at Cambridge, Wardlaw and McGuire (1931) at Trinidad and Hirai (1938) from Formosa deal however mainly with transport and storage problems. Preli-

minary surveys made in England by Meridith (1960) revealed that in some cases wastage caused by the fungus had affected the complete shipment which may vary between 70,000 to 130,000 stems.

The observation made in the present study revealed that in Madras State, the disease was prevalent to a large extent in the field causing a rot of very young fruits and fruits starting to ripen. The prevalence of the disease in India was first recorded by Dastur (1916) who reported that the disease occurred in the field and to a certain extent in storage. Serrano (1925) also reported the anthracnose or fruit rot as a common disease in India. Chona (1933) reported that the disease occurred in the Punjab markets, fruit stalls, curing pits and store houses at Lyallpur, Lahore and Jhelum. The survey made by him revealed that a high percentage of bananas mostly imported were also affected by the disease, showing that these are most probably present in other banana growing parts of India as well. Subsequently it was observed that the disease occurred in the banana plantation in Amritsar and Lahore, considerably retarding the development of the fruit and weakening of the main stalk causing the main plant and the entire bunch sometimes being blown by strong wind. Jain (1950) also observed the disease in India not only on ripe fruits in storage or transit but also occassionally affecting the young immature unreaped fruits in the field on the crop at Sagar in Madhya Pradesh. Roy and Sharma (1952) reported in Bihar ripe fruits being affected by *G. musarum*.

Symptomatology:

In the present study a variety of symptoms involving all parts of the banana bunch has been observed. The symptoms observed by Cobb (1906) were that in fruits of 3″ or less in length, there was a general discolouration accompanied by shrivelling. The colour changed through shades of green, yellow, brown and finally black. The final blackening was accompanied by pink eruptions of *Gloeosporium* acervuli. The above symptoms were observed by the author on the varieties *Peykunnan* and *Monthan*.

Dastur (1916), Agati (1922) and Serrano (1925) observed that the fungus caused distinct black depressed lesions on the fruit with the lesions coalescing and eventually covered the whole fruit causing premature ripening and shrivelling of the fruits on which were found pink spore beds of the fungus. Simmonds (1928)

observed that in addition to the above the fungus also invaded the pulp causing a soft rot. The varieties under study namely *Musa balbisiana* (Aduthurai), *Musa balbisiana* (Kallar), *Krishnavazhai* and *Vayilvazhai* also exhibited the above symptom. Chona (1933) observed that the disease at first caused a stalk rot. This rot originated as a dark brown patch on the central point of the main curvature of the main stalk. The rot gradually spread downwards to the fruits retarding the development of the fruits and weakening the main stalk. This symptom is in agreement with the symptom observed on the variety *Kuribontha* and *Malavazhai* in the present study.

However, some of the symptoms observed in this study have not been recorded so far, as in the case of *Rasthali, Karivazhai, Walha, Nendrapadathi, Peyan, Pachanadan, Kadali, Ayiramkarasthali, Virupakshi* and *Neyvazhai,* where there was the browning and blackening of mature and immature fruits without any descrete lesions. Another symptom of importance and not recorded was the type, where there were no blackened lesions, instead acervuli were found on water soaked oily lesions as in *Kaali* and *Peyladen.* In the case of *Vellavazhai* (Coimbatore), *Padathi, Peyan* (Tirunelveli), *Vellavazhai* (Pannakad) and *Aattukomban,* the blackening of the whole bunch in a short time followed by shrivelling and drying without the formation of acervuli was also another kind of symptom observed.

Morphology of the fungus on the fruit:

Much of the study on the morphology of the fungus such as nature of the mycelium whether inter or intracellular, various developmental stages in the formation of acervuli, namely stroma, conidia, their size and shape etc. have already been described by various workers as Toro (1922), Agati (1922 and 1925) and Jain (1950). Toro (1922) described eleven strains of the fungus differing in spore measurements from Puerto Rico. He found the largest form measured 15-25 $\times$ 4-6 μ and the smallest ranged from 10-13 $\times$ 3-4·5 μ. Kruger (1913) who isolated an ascosporic strain from ripe fruit imported into Germany found the conidia in the fruit to measure 15-17·5 $\times$ 4·6-6 μ. It is clear therefore that the measurements of the conidia of the fungus are very variable.

The results of present study also confirm the above findings. It was observed that in the 26 isolates under study the spore measurements ranged from 9·8-14 $\times$ 4·2-6·7 μ to 9·8-29·4 $\times$ 4·9-7 μ.

These two extremes were connected by a series of isolates showing intergrading measurements. The mean measurements however, varied only within a narrow range of 9·8-14 μ. There was very little variation in conidial widths.

The relationship of morphological and chemical characteristics of fruit to susceptibility:

Agati (1922) found that sweeter varieties required 6 to 10 days for infection and the less sweet varieties required 15 to 20 days. This conclusion is also supported by Toro (1922) who found that the fruits of Dwarf or Canary banana (*M. cavendishi*) were more susceptible than those of plantain (*M. paradisiaca*). By "infection" these authors presumably meant the first appearance of an evident lesion. In the present study however, the size of lesion on a given day is taken as a measure of susceptibility as it is considered that the extent of rot caused in a given period would be a more accurate criterion. It is also not clear from the accounts of these authors what exactly was meant by "sweet" and less sweet varieties as they have not attempted a quantitative correlation between sugar content and susceptibility. According to Stratton and von Loesecke (1930), the sweeter varieties like Gros Michel contain a greater amount of reducing sugar than the less sweet varieties like plantain. It is also well known that sweetness of any variety of banana is contributed by its reducing sugar contents.

In this paper the relationship of reducing sugars and sucrose content of 20 varieties of fruits to susceptibility was studied. The diameter of the lesion and the length of the incubation period were noted in all the 20 varieties when inoculated with the four isolates. The correlations between reducing sugar contents, sucrose contents and diameter of lesion and incubation period were worked out. It was observed that the reducing sugar content of the fruit was positively correlated with diameter of lesion, i.e. those varieties which had higher reducing sugar content developed larger lesions in a shorter time. In these varieties the acervuli were formed much later than in those having lesser reducing sugar content. It may be mentioned here that once the acervuli have formed, the lesion does not enlarge any further.

On the other hand, it was found that the sucrose content was negatively correlated with diameter of lesions and incubation period i.e., varieties having a higher sucrose content developed smaller lesions but the fructification appeared much earlier on these

lesions. It will be obvious that a rapidly advancing lesion causes greater damage to the fruit than a slowly expanding lesions. But in the latter case, there is a possibility of fresh lesions being formed by new infection caused by conidia already produced on the earlier lesions. The present study has therefore, helped to pinpoint the exact component of the "sweetness" mentioned by the earlier authors as responsible for susceptibility. It is shown here that the reducing sugar contents contributes to the expansion of the lesion.

Infection in relation to the age of fruit:

Earlier workers have noticed that although anthracnose symptoms are seen on ripe fruit in storage, infection occurs during flowering and the formation of young fruits—Simmonds (1941); Dastur (1916); Vietch (1941), Baker and Wardlaw (1937); Wardlaw and McGuire (1931, 1932); Meridith (1960); Chakravarthy (1957). The "latent infection" theory was first proposed by Dastur (1916) and it was later confirmed by Simmonds (1941). Baker and Wardlaw (1937) detected the presence of the fungus within the rind tissue of mature and immature fruits. Further histological evidence of latent infection was provided by Chakravarthy (1957). She found that when green Gros Michel bananas were inoculated, the fungus penetrated the cuticle mechanically. The fungus thereafter remained in an inactive stage in a subcuticular position until the ripening of the fruit began. The above is the general pattern of behaviour observed by most of the workers referred to above. However Dastur (1916) showed that occassionally in India immature green fruits took infection and became immediately diseased, without the latent period in the field.

In this paper, the inoculation experiments conducted on four varities of banana at 2 day interval showed that the type of behaviour discussed above is found only in certain varieties. The variety *Vellavazhai* did not develop any lesion up to 68th day from the time of flower opening. Thereafter it became susceptible and readily developed lesions when inoculated with the fungus. The variety therefore, behaved exactly like the Gros Michel variety referred to by Chakravarthy (1957) and others in having a latent period till fruit attained maturity. The two other varieties i.e., *Kuribontha* and *Peyan* behaved in an entirely different way. These varieties were highly susceptible in the young and immature stage but became nonsusceptible for varying periods and again became susceptible when the fruits attained full maturity. The variety *Kaali* behaved somewhat differently. It had an early

susceptible period up to 35 days and then a short period of susceptibility of 30 days and became again resistant for a long period of 57 days and thereafter it became susceptible at maturity. It is not clear however, from the present experiment whether the fungus remained latent during the nonsusceptible period. But it is clear that the type of behaviour observed in Gros Michel by the earlier authors and in *Vellavazhai* in the present study is not always the rule. This study has also shown that the observation of Dastur (1916) where he found immature fruits in the field being infected, is more common than was formerly supposed.

Another interesting observation made in this study was the variation in incubation period at various stages of growth of banana fruit. In *Peyan* initially the incubation period was 9 days which rose up to 17 days till the fruits became nonsusceptible. At maturity, it again took infection with a longer incubation period of 20 days which fell to 10 days as the fruits got ripened.

In the case of *Vellavazhai,* initial infection was noticed with an incubation period of 16 days. But at ripening stage it fell to 8 days.

In *kaali,* the incubation period was 13 days to start with, then it rose to 20 days. Thereafter the fruits became nonsusceptible. At maturity the fruits again got infected within 13 days and then it fell at ripening stage to 10 days.

As regards *Kuribontha,* the incubation period was 8 days, then it rose to 16 days and then the fruits became nonsusceptible. Again it took infection within ten days and the incubation period fell to 8 days at the ripening stage.

Pathogenicity and varietal resistance:

Agati (1922) found that the 30 varieties he examined showed some degree of infection by *G. musarum.* The greatest amount of infection noticed was on sweet varieties including *Lacatan, Bongolan* and *Cavendish.* The less sweet fruits were not so subject to attack. He divided the fruits into two classes (1) those readily infected with 6-10 days and (3) those less readily infected in 15-23 days. Toro (1922) confirmed Agati's observation and stated the Dwarf or Canary banana (*M cavendishi*) as most susceptible, being a sweet variety and that plantain varieties *M. paradisiaca* showed greater degree of resistance. Rios (1930) stated that in Puerto Rico, the variety more susceptible is *Guino enana.* According to Park (1933) the disease is most common on the cooking

variety known as ash plantain (*S. aluchel*). Parham (1935) observed *G. musarum* causing a severe finger stalk and fruit spotting of the veimama and cavendish varieties. Roy and Sharma (1952) state that ripe fruits of *Sabja* variety are attacked by *G. musarum* throughout the State of Bihar. Chakravarthy (1957) observed Cameroon *Gros Michel* banana susceptible to *G. musarum* when artificially inoculated.

Tomkins (1931) introduced into small wounds on sound sterilised bananas, mycelium and spores of *Fusarium* and *Gloeosporium*. Typical finger rot invariably followed the inoculation of the bananas with either of these fungi. Wardlaw (1931) in order to test the pathogenicity of *G. musarum* in the field inoculated by wounding younger fruits representing three stages of development namely (a) very young fingers just after emergence of fingers (b) slightly older when the perianth had commenced to wither and (c) fingers on a half grown bunch; but negative results were obtained throughout. Jain (1950) tested the pathogenicity of *G. musarum* in the laboratory on the plantain fruits of various stages of development by wounding and atomising the spore suspension of the organism in water. The very young fruits 1" to 2" in length with perianth still attached to them, showed the symptom of infection in 4 to 5 days and became rotted completely in 3 to 6 days. Fruits of comparatively advanced stage 4-6" long could be infected only through wounds or bruises and the incubation ranged from 7 to 15 days.

In the present study 31 varieties of banana were tested for their reaction to the pathogen. It was observed that four varieties namely *Thellabontha, Kunnan, M. chiliocarpa* and *Malavazhai* were infected by all isolates and one namely *Boodithamontha bathees* was affected only by four isolates. It was further observed that one particular isolate, i.e., *Kaali* isolate when inoculated on all varieties showed varying incubation period ranging from 5 to 25 days.

Similarly a great variation in the range of pathogenecity of the isolates were observed. The isolate from *Kuribontha* was found to be most virulent affecting 29 varieties. The isolate from *Peykunnan* was found to be the least virulent since it infected only 13 varieties.

All the isolates also showed varying incubation period when inoculated on a particular variety namely *Kaali*. On *Kaali* an

incubation period ranging from 5-9 days was exhibited by all the isolates.

Table V shows the nature of fruits when ripe. Most of the varieties tested had sweet fruits except *Thellabontha, Musa chiliocarpa, Musa balbisiana, Kuribontha, Padathi, Monthan,* and *Booditha montha bathees* which had insipid fruits. The percentage of isolates infecting each variety is also given in the Table. It is observed that all the varieties are highly susceptible. But it is not possible to say that the insipid varieties are resistant as some of the insipid varieties like *Padathi* and *Monthan* have been infected by 69.2 and 57.7% of the isolates. The two species of *Musa, M. chiliocarpa* and *M. balbisiana* which have very insipid fruits were infected by 100% and 92·3% of the isolates respectively. It must however, be pointed out that the very insipid variety *Booditha montha bathees* was infected by only 15·4% of the isolates. The results therefore, indicate that it is not possible to make a generalisation that the sweeter varieties are more susceptible than the insipid varieties. It can however be stated that the sweet varieties are always highly susceptible. But some insipid varieties are also highly susceptible.

According to Simmonds (1959) the majority of edible bananas had their origin only in two wild species—*Musa acuminata* and *M. balbisiana.* The cultivars derived from the parent species may be diploids, triploids or tetraploids and may differ in the relative genomic contents derived from the two parents. Several groups of cultivars have been recognised by Simmonds based on ploidy and genomic compositon. The groups are designated by letters which designate their genomic composition with respect to the two parent species (A=*M.acuminata* and B=*M balbisiana*) as well as their ploidy. Thus for example AA would indicate that the cultivar is a diploid with genomes derived solely from *M acuminata,* while AAB indicate that it is a triploid with two genomes from *M.acuminata* and one from *M.balbisiana.*

The cultivars whose susceptibility to infection by *G.musarum* have been studied are arranged according to Simmonds system of classification. The group designations are indicated against the varieties in Table V which gives the range of suceptibility of the varieties tested. It is seen that there is no correlation between the genomic composition or ploidy of a variety and its range of susceptibility.

Table VI also indicates the incubation periods of the 26 isolates on the 31 varieties. Though the incubation period varied quite considerably, no sort of correlation could be observed between the genomic composition of the variety and the length of incubation period.

SUMMARY

1. A survey of anthracnose disease of banana in Madras State revealed that all important varieties of banana were affected. The incidence was found to range from 10-15%.

2. In the present study a variety of symptoms involving all parts of the banana bunch has been observed. Some of the symtoms observed in this study have not been recorded so far.

3. Twenty six isolates of the pathogen were studied with particular refrence to variations in morphology pathogenicity and varietal infection.

4. The measurements of the conidia of the fungus was found somewhat variable in the 26 isolates studied. The mean measurements varied only within a narrow range 9·8-14 μ. There was also very little variation in the conidial width.

5. The varieties which had higher reducing sugar content developed larger lesions in a shorter time, but the fructification was much belated. On the other hand varieties having higher sucrose content developed smaller lesions but the fructification appeared much earlier.

6. As regards infection in relation to age of the fruit the variety *Vellavazhai* developed lesions from 69 day up to the ripening stage. *Kuribontha* and *Peyan* were highly susceptible in the young and immature stage and also when the fruits attained full maturity The variety *Kaali* was susceptible after 25 days, for a short period and then again at maturity.

7. Another interesting observation made in this study was variation in the incubation period exhibited by four isolates i.e., from *Peyan, Vellavazhai, Kaali* and *Kuribontha* on their respective hosts.

8. There was a great variation in the range of pathogenicity of the isolates. The isolate from *Kuribontha* affected maximum number of varieties namely 29 and *Peykunnan* isolate the least

namely thirteen. In general, all the isolates required a long incubation period of 16-25 days on varieties *M.balbisiana* and *Raja vazhai*, while the isolates on *Nendrapadathi* took a minimum incubation period of 4-7 days; 5-9 days on *Kaali*, 5-7 on *Malavazhai* and 5-9 *Kunnan*.

9. Out of 31 banana varieties tested for their reaction to the pathogen, four varieties namely *Thellabontha, Kunnan, M. chiliocarp* and *Malavazhai* were infected by all isolates and one namely *Boodithamonthabathees* was affected only by four isolates. It was further observed that the varieties when inoculated with a particular isolate, showed varying incubation period.

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Generalized Metric Lattices I—The Bm-Lattices

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ABSTRACT

This paper deals with a generalization of the notion of the classical metric lattice obtained by replacing the real line by a suitably chosen Boolean algebra These lattices, called the Bm-lattices, are completely characterized and a natural class of uniformities and their completions on these Bm-lattices are studied. The paper also contains some results on the "congruent mappings" on Bm-lattices.

I *Preface*: In this paper we introduce the notion of a Boolean valued modular functional — a generalization of the classical real valued modular functional — in a lattice and obtain the necessary and sufficient condition for a lattice L to admit a positive Boolean valued modular functional into a suitably chosen Boolean algebra B. A lattice L admitting such a functional m — which we call a Bm-Lattice — is shown to be a set admitting a Boolean metric d (cf[2]*) into B. We study the Boolean uniformities on L (which notion has been introduced in [3]) defined by d. The completions of the Boolean uniformities are also seen to be Bm-lattices, the uniformity of the completion being a corresponding Boolean uniformity. The paper ends with the study of the "congruent mappings" on Bm-lattices.

Here $\dotplus$, . and / will stand for the lattice sum, lattice product and complementation in lattices respectively and $\oplus$ will denote the symmetric difference operation in Boolean algebra.

II. *The B-modular functional*: Here we shall introduce the notion of a Boolean valued modular functional on a lattice L and study its general properties.

*The numbers in square brackets refer to the Bibliography at the end of this paper.

Definition 2.1. A mapping m of a lattice L into a Boolean algebra B is said to be a Boolean valued modular functional (which we call a B-Modular functional, where B stands for the Boolean algebra with respect to which m is defined) if it satisfies the following condition:

$$m(a+b) \oplus m(ab) = m(a) \oplus m(b) \text{ for all } a, b \in L.$$

If further, for $a > b$ in L, $m(a) > m(b)$ in B then the B-modular functional m is said to be positive. A lattice L which admits a positive B-modular functional m (into a suitably chosen Boolean algebra B) is said to be a Bm-lattice.

Lemma 2.1. In a Boolean algebra B the symmetric difference of any two elements is $\leqslant$ their lattice sum.

Proof: Let $a, b \in L$. Then $a \oplus b = ab' + a'b \leqslant a + b$.

We have

Proposition 2.1' Let L be a Bm-lattice. Then the B-modular functional m is a lattice isomorphism of L in B.

Proof: We will first show that m is (1—1). Let $m(x) = m(y)$. Then $m(x+y) \oplus m(xy) = m(x) \oplus m(y) = m(x) \oplus m(x) = 0$. Hence $m(x+y) = m(xy)$ .. .. (I).

Since $x+y \geqslant xy$, if $x \neq y$ then $x+y > xy$. From the positivity of m, it follows that $m(x+y) > m(xy)$ contrary to (I). Hence $x=y$. Therefore m is (1—1).

We will next show that m preserves lattice sums and products.

Now $m(x+y) = (m(x) \oplus m(y)) \oplus m(xy)$

$\leqslant (m(x) \oplus m(y)) + m(xy)$ (cf. lemma 2.1)

$\leqslant m(x) + m(y) + m(xy)$ (cf. lemma 2.1)

$= m(x) + m(y)$ (as m is positive) ..... (II).

Since $x+y \geqslant x$, y and m is positive $m(x+y) \geqslant m((x)$ and $m(y)$. Therefore, $m(x+y) \geqslant m(x) + m(y)$. Therefore from (II) we deduce that $m(x+y) = m(x) + m(y)$ .. .. (III).

Again $m((xy) = m(x+y) \oplus m(x) \oplus m(y)$

$= (m(x) + m(y)) \oplus m(x) \oplus m(y)$ (From (III)).

$= (m(x))' m(y) \oplus m(y)$

$= m(x).m(y)$.

Therefore m is an isomorphism of L in B.

As an immediate consequence of this result we have

Corollary 2.1: Any Bm-lattic is distributive.

The following proposition is the converse of Corollary 2.1.

Proposition 2.2: Any distributive lattice L is a Bm-lattice.

Proof: L being distributive, can be imbeded as a sub lattice of a Boolean algebra B (cf [1]). Let f denote this isomorphism of L in B. For $a \in L$ define $m(a) = f(a)$. Then $m(a+b) \oplus m(ab) = f(a+b) \oplus f(ab) = (f(a)+f(b)) \oplus f(a)\, f(b) = (f(a)+f(b))\,[(f(a))'+(f(b))'] = f(a) \oplus f(b) = m(a) \oplus m(b)$. Further as f is (1-1) m is positive. Thus L is a Bm-lattice.

Thus we have

Proposition 2.3: The Bm-lattices are precisely the distributive lattices.

Now we shall proceed to study the properties of the positive B-modular functional m on a Bm-lattice L. We begin by recalling the following definition from [2].

Definition 2.2: Let S be any set. A mapping d of the product set $S \times S$ into a Boolean algebra B is said to be a B-metric on S if it satisfies the following conditions:

(1) $d(a,b) = O \ (=) \ a = b$.

(2) $d(a,b) = d(b,a)$.

(3) $d(a,b) \leqslant d(a,c) + d(c,b)$ (for all $a,b,c \in S$).

We now have

Lemma 2.2: In a Bm-lattice L the following are true:

(1) $d(a,b) = m(a+b) \oplus m(ab)$ is a B-metric on L. (this we call the B-metric determined by m).

(2) $d(a,c) = d(a,b) \oplus d(b,c)$.

(3) $d(a+x, b+x) \oplus d(ax, bx) = d(a,b)$.

(4) $d(a+c, b+d) \leqslant d(a,b) + d(c,d)$.

(5) $d(ac, bd) \leqslant d(a,b) + d(c,d)$.

Proof: Using the fact that m is a lattice isomorphism these statements can easily be proved.

III. *The Bm-uniform structures*: It can easily be verified that given a dual ideal P of B the subsets $A_p = [(x,y)/d(x,y) \leqslant$

p] ($p \varepsilon P$) form a base for a uniformity* A_P on L. Each A_P is symmetric and further $A_p \circ A_p = A_p$. The "Boolean uniformity" A_P is called the (B,d,P)—uniformity on L, where B is the Boolean algebra into which m is defined, d is the B-metric on L defined by m (cf. lemma 2.2 (1)), and P is the dual ideal defining A_P. In particular if L = B and if m(a) = a then $d(a,b) = a \oplus b$ is called the "auto-metric" on B (cf. [3]). In this case A_P is called an auto-uniformity on B.

Hereafter (L, A_P) will denote a Bm-lattice L together with the (B, d, P)—uniformity A_P, d being the B-metric determined by m.

We have

Proposition 3.1: Let L be any Bm-lattice and let P be any dual ideal of B. Then $(L\ A_P)$ is isomorphic and unimorphic to a sublattice of (B, U_P), U_P being the auto uniformity defined by P on B.

Proof: From prop. 2.1 it follows that L is isomorphic to a sublattice of B, m being the isomorphism. Since $(x,y) \varepsilon A_p (=) m(x) \oplus m(y) = (m(x+y) \oplus m(xy)) \leqslant p (=) (m(x), m(y)) \varepsilon U_p$, it follows that m is a unimorphism.

We also have

Proposition 3.2: The lattice sum and product in L are uniformly continuous with respect to the uniformity A_P.

Proof: This follows from lemma 2.2 (4 and 5).

IV. *Completion of a Bm-uniform lattice*: In this section we study the uniform completion of (L, A_P). We begin with

Definition 4.1: Let P be any dual ideal of B. Then the subset $C(P) = [b \varepsilon B / b \leqslant$ each $p \varepsilon P]$ is said to be the cut complement of P in B.

Then we have

Proposition 4.1: Let L be a Bm-lattice and let P be any dual ideal of B with cut complement zero† and let $(B^* U_P^*)$ be the uni-

* In fact these uniformities can be defined on arbitrary sets admitting Boolean metrics. An exhaustive study of these uniformities, called "Boolean uniformities", has been made in [3].

†This ensures that (L, AP) and (B, UP) are Hausdorff. The existence of such (proper) dual ideals in infinite Boolean algebras is known.

form completion of (B, U_P) (where U_P is the auto uniformity defined by P on B). Then the completion $(L^* A_P^*)$ of $(L A_P)$ is a B*m*-lattice, A_P^* being the Boolean uniformity defined by the B*-metric determined by m* and the dual ideal generated by P in B*.

Proof: Let (a_p), (b_p) be Cauchy- P-nets of L (where P as an index set is ordered as follows: $p \succ q$ in $P(=)A_p \subseteq A_q$). It follows from lemma 2·2 (4 and 5) that $(a_p + b_p)$, $(a_p b_p)$ are also Cauchy-P-nets of L. Further if (a_p) is equivalent to $(b_p$ and (c_p) is equivalent to (d_p) then for each $p \varepsilon P$ there exist $d_1(p)$, $d_2(p)$ εP such that $(a_i, b_j) \varepsilon A_p$ for all i, $j \succ d_1(p)$ and $(c_j, d_i) \varepsilon A_p$ for all i, $j \succ d_2(p)$. Let $d(p)$ be $\succ d_1(p)$, $d_2(p)$. Then for all i, $j \succ d(p)$, $d(a_i + c_i, b_j + d_j) \leqslant d(a_i, b_j) + d(c_i, d_j)$ (from lemma 2.2 (4)) $\leqslant p + p = p$. i.e., $(a_i + c_i, b_j + d_j) \varepsilon A_p$ for all i, $j \succ d(p)$. Hence $(a_p + c_p)$ is equivalent to $(b_p + d_p)$. Similarly $(a_p c_p)$ is equivalent to $(b_p d_p)$.

Let $a^*, b^* \varepsilon L^*$. Then a^*, b^* are classes of equivalent Cauchy-P-nets containing the Cauchy-P-nets (a_p), (b_p) of L respectively. Define $a^* + b^*$, $a^* b^*$ as the classes $\{(a_p + b_p)\}$, $\{(a_p b_p)\}$ of Cauchy-P-nets containing $(a_p + b_p)$, $(a_p b_p)$. Then from the above observations we see that L^* is a lattice with respect to these operations.

Next let $a^* = \{(a_p)\} \varepsilon L^*$. Consider the P-net $(m(a_p))$ of (B, U_P). This obviously is a Cauchy-P-net in (B, U_P) and determines therefore an element $m^*(a^*)$ in B^*. If (a_p) is equivalent to (b_p) then $(m(a_p))$ is equivalent to $(m(b_p))$. Therefore $m^*(a^*) = m^*(b^*)$. $m^*(a^*) \oplus m^*(b^*) = \{(m(a_p))\} \oplus \{(m(b_p))\} = \{(m(a_p) \oplus m(b_p))\}$ (as (B, U_P) is a topological group with respect to $\oplus$) $= \{(m(a_p + b_p) \oplus m(a_p b_p))\} = \{(m(a_p + b_p))\} \oplus \{(m(a_p b_p))\} = m^*(a^* + b^*) \oplus m^*(a^* b^*)$. Further if $a^* > b^*$ then $a^* + b^* = a^*$. i.e., $(a_p + b_p)$ and (a_p) are equivalent (where $a^* \ni (a_p)$, $b^* \ni (b_p)$). Therefore, $(m(a_p + b_p))$ and $(m(a_p))$ are equivalent in L i.e., $(m(a_p)) + (m(b_p))$ and $(m(a_p))$ are equivalent in B i.e., $m^*(a^*) + m^*(b^*) = m^*(a^*)$. Therefore, $m^*(a^*) \geqslant m^*(b^*)$. If $m^*(a^*) = m^*(b^*)$, then $(m(a_p))$ is equivalent to $(m(b_p))$ and hence (a_p) is equivalent to (b_p) i.e., $a^* = b^*$, contrary to assumption. Therefore, $m^*(a^*) > m^*(b^*)$. Thus m^* is a positive B* -modular functional on L.* Further $(a^*, b^*) \varepsilon A_p^*$ (where A_p^* is the member corresponding

to A_p in the uniformity for L*) (=) there exists $d(p) \in P$ such that $(a_i, b_j) \in A_p$ for all $i, j \propto d(p)$ (=) $(m(a_i), m(b_j)) \in U_p$ for all $i, j \propto d(p)$ (=) $(m^*(a^*), m^*(b^*)) \in U_p^*$ (where U_p^* is the member corresponding to U_p in the uniformity for B*). But it can easily be verified that $U_p^* = U_{p^*}$ where p^* is the repeated Cauchy-P-net determined by p on B. Let P* be the dual ideal generated by P in B*. If $q \in P^*$, then $q \geqslant$ some p^* $(p \in B)$. Therefore it follows that U_p^* is equivalent to U_{p^*} Consequently we have that the (L*, d*, P*)—uniformity on L* is equivalent to its completion uniformity (d* being the B* -determined by m* on L*) and this proves the result.

We will now recall the following definition from [3].

Definition 4.2: Let (L, V) be a lattice with a uniformity V. Then V is said to be a congruence uniformity on L if there exists a base $[V_i]$ for V such that to each V_i there exists a congruence θ_i on L such that $(x, y) \in V_i$ (=) $x \theta_i y$.

We have

Proposition 4·2: Let L be a Bm-lattice. Then A_p is a congruence uniformity on L.

Proof: For each $p \in P$ define $x \theta_p y$ (=) $d(x, y) \leqslant p$. Then θ_p can easily be verified to be a congruence relation on L. Since for each $p \in P$, $Ap = [(x, y)/x \theta_p y]$ it follows that A_p is a congruence uniformity on L.

An an important consequence of this we have the following result which is a particular case of a more general theorem in [3].

Proposition 4·3: Let L be a Bm-lattice and let P be any dual ideal of B with cut complement zero. Then (L, A_p) can be uniformly imbedded as a dense sub lattice of the projective limit of the discrete quotient lattices $L_p = L/\theta_p$, $p \in P$; where for each p, θ_p is the congruence determined by p as in prop. 4·2.

*Proof**: For $p \propto q$ (i.e., $A_q \subset A_p$) define the map φ_q^p of L_q on L_p as follows: $\varphi_q^p[(a)_q] = (a)_p$ (where $(a)_q$ is the congruence class containing $a \in L$ with respect to θ_q). Then $[A_p, \varphi_q^p]$, $(p \in P)$, can be verified to be an inverse mapping system of lattices (each with the "discrete" uniformity i.e., $\triangle$ is the only surround-

* This has been proved in detail in [3].

ing of the diagonal). Let L^* be their projective limit. Define a mapping φ of L in L^* as follows: $\varphi(a)=[(a)_p]$ $(p \in P)$, for any $a \in L$. Then $\varphi(L)$ can be verified to be a dense sublattice of L^* and φ can be verified to be a unimorphism.

Corollary 4.1: The completion of (L, A_p) is the projective limit of the discrete factor lattices L/θ_p, $(p \in P)$.

V. *The congruent mappings of a Bm-lattice in B*: In this section we shall study the "congruent mappings" on a Bm-lattice L.

Let L be a Bm-lattice with the associated Boolean algebra B. Let d be the B-metric on L determined by m and let d_1 be the auto-metric on B. Then

Definition 1.5: A mapping f of L in B is said to be a congruent mapping if $d(a, b) = d_1(f(a), f(b))$ for all $a, b \in L$.

Lemma 5.1: The congruent mappings of L in B are precisely those mappings of the form $f(x) = m(x) \oplus a$, where $a \in B$.

Proof: Consider $f(x) = m(x) \oplus a$ for some $a \in B$. f is a congruent mapping since $d(x, y) = m(x) \oplus m(y) = m(x) \oplus m(y) \oplus a \oplus a = (m(x) \oplus a) \oplus (m(y) \oplus a) = f(x) \oplus f(y) = d_1(f(x), f(y))$.

Conversely let f be some congruent mapping on L. Then $d(x, y) = d_1(f(x), f(y))$ for all $x, y \in L$. Hence $m(x) \oplus m(y) = f(x) \oplus f(y)$ for all $x, y \in L$. Therefore, $m(x) \oplus f(x) = m(y) \oplus f(y)$. This is an element a of B independent of x, and $m(x) \oplus f(x) = a$ i.e., $f(x) = m(x) \oplus a$ whatever be $x \in L$ and hence the result. We have

Proposition 5.2: The set G(L) of all congruent mappings of L in B form a group which is isomorphic to the additive group of B.

Proof: For $f, g \in G(L)$ define fg as follows: $(fg)(x) = f(x) \oplus g(x) \oplus m(x)$. From lemma 5.1 $f(x) = m(x) \oplus a$, $g(x) = m(x) \oplus b$, for some $a, b \in L$. Hence $(fg)(x) = m(x) \oplus a \oplus m(x) \oplus b \oplus m(x) = m(x) \oplus a \oplus b$. Therefore, by lemma 5.1 $fg \in G(L)$. Again $(fm)(x) = f(x) \oplus m(x) \oplus m(x) = f(x)$ for all $x \in L$. Therefore $fm = f$ for all $f \in G(L)$. Further $(ff)(x) = f(x) \oplus f(x) \oplus m(x) = m(x)$, for all $x \in L$. Thus m is the identity on G(L) and each f in G(L) is its own inverse and consequently G(L) is a group.

For $a \varepsilon B$ define $\varphi(a) = f_a \varepsilon G(L)$ where f_a is the mapping $x \rightarrow m(x) \oplus a$. This can easily be verified to be an isomorphism of the addtive group of B on G(L) an dthis completes the proof.

In particular we have

Corollary 5.1: Let B be an auto metrized Boolean algebra. Then the congruent mappings of B in itself are precisely those of the form $f(x) = x \oplus a$ for $a \varepsilon B$. These form a group isomorphic to the additive group of B.

In conclusion I wish to express my thanks to Dr. V. S. Krishnan, Professor of Mathematics, University of Madras and Dr. V. K. Balachandran Reader in Mathematics, University of Madras for their kind help and valuable guidance during the preparation of this paper.

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The Anatomy of the Crab Neptunus Sanguino-Lentus Herbst

Part IV: Reproductive system and embryological studies*

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ABSTRACT

The male and female reproductive organs of the crab *Neptunus sanguinolentus* Herbst are described. The absence of spines at the tip of the genital papilla which remain outside the pleopod in immature specimens, the presence of both three rayed and four rayed spermatozoa, the posterior extremities of the two halves of the ovary remaining separate throughout the life of the crab, the presence of the sperm plug inside the spermatheca of soft and recently copulated females are some of the interesting features in the reproductive system of this crab.

The embryonic development during the three days before hatching is also described.

Introduction

The following contribution formed part of a study of the complete anatomy of the common edible crab of the Madras coast *Neptunus sanguinolentus* Herbst (The generic name revised to *Portunus* according to Stephenson & Campbell, 1959). It is in continuation of the previous accounts (George, 1961 a, b and c) and as in those papers emphasis is given to the points which differ from the European form *Cancer pagurus* described by Pearson (1908).

I wish to express my thanks to Dr. C. P. Gnanamuthu for his help and guidance and the Syndicate of the Madras University for the award of a studentship during the tenure of the work.

* Edited from a thesis approved for M.Sc. degree.

Reproductive System

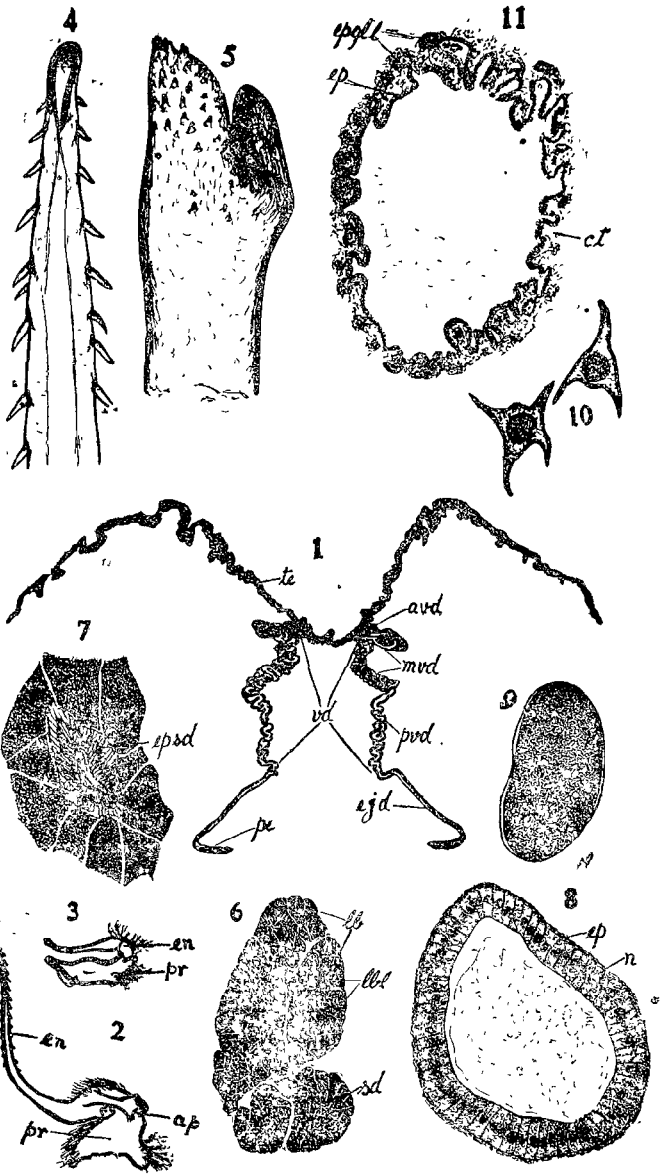
Among the many authors who have worked on the reproductive organs of the Brachyura the names of Fasten, Bhattacharya, Nath, Muthuswamy Iyer, Spaulding and Cronin may be mentioned. Fasten (1918 & 1924) has done extensive work on the spermatogenesis of several *Cancer* crabs. He also described the male reproductive organs of crabs. Bhattacharya (1931) studied the oogenesis of the crab *Scylla serrata* and Nath (1932) and Muthuswamy Iyer (1933) the spermatogenesis of the crabs *Paratelphusa spinigera* and *Paratelphusa hydrodromus* respectively. Spaulding (1942) described the female reproductive organs and the formation of the sperm plug inside the spermatheca of *Carcinus maenas*. The male reproductive organs and the organs of copulation are also described by him. Cronin (1947) has done the complete anatomy of the male organs of the blue crab *Callinectes sapidus* and has given minute histological details of all the parts. Van Engel (1958) reported on the reproduction of the same species from Chesapeake Bay.

Male. (Text-Fig. 1-11).

As in other Brachyura the adult male can be easily distinguished from the female by the shape of the abdomen and the fusion of the abdominal segments. In addition mature males have larger and broader chelae. The first and second abdominal appendages are highly modified to form an intromittent copulatory organ.

The first pair is larger than the second, with a long rod-like endopodite which is four times longer than the stout basal protopodite from which it starts (Text-Fig. 2). This rod-like structure is quite straight at its tip and has no sharp bend at that place like that of some other crabs. It has an aperture on the dorsal side at the junction between it and the protopodite. This aperture leads forwards, so that the endopodite is tubular and at the tip there is a longitudinal slit-like opening as shown in Text-Fig. 4. The margins of its distal half are beset with somewhat stout spines directed backwards. On the ventral side of the base of the first pleopod also there is a slit-like opening into which is fitted the tip of the genital papilla in mature crabs.

In the second pair of pleopods (Text-Fig. 3) which arise behind the first pair from the second abdominal sternum there are



TEXT-FIGS. 1-11.

two parts, a protopodite and an endopodite, both of which are placed in a horizontal position so that in the natural position the tip of the rod-like endopodite fits into the opening on the dorsal side of the first pleopod. The apex of this rod-like endopodite is slightly swollen and transparent and is forked into two blunt unequal forks (Text-Fig. 5). Spiny projections are present on the larger of the two. In *Carcinus maenas* there is a small blade-like process arising from the tip of the endopodite instead of the fork (Spaulding, 1942). The endopodite is of the same length as the anterior length of the protopodite. Plumed setae are present along the anterior edge of the protopodite and the base of the endopodite.

The posterior appendage reaches only just in front of the base of the long tube of the anterior one. In mature specimens the tip of the posterior appendage can be seen inserted into the posterior foramen at the base of the anterior appendage. The genital papilla or penis extends from the base of the coxopodite of the last walking leg upto the base of the first abdominal appendage on the anterior side and the tip of it is inserted in the anterior foramen of the appendage. The musculature of the two pairs of appendages is identical with that of *Callinectes sapidus* described by Chochran (1935).

The male reproductive system consists of the paired testes, vasa efferentia, vasa deferentia with the anterior, median and posterior portions, ejaculatory duct and the external penis (Text-Fig. 1).

The testes of *Neptunus sanguinolentus* are constructed on the same plan as described by Pearson (1908), Binford (1913), Fasten (1915 & 1918) and Cronin (1947). It is a paired organ having the appearance of a slender white convoluted tube, 2 to 3 mm. in average width and lying beneath the dorsal wall of the carapace on the antero-dorsal surface of the digestive gland. Each extends from behind the dorsal region of the pyloric gut, passes along the sides of the foregut forwards and outwards and reaches the anterior corner of the antero-lateral region. From there it passes backwards and outwards along the antero-lateral region and ends near the basal region of the great lateral spine. A short cross bar joins the testes of the two sides behind the pyloric foregut and in front of the heart. The spermatic artery which is a branch of the lateral artery is visible along the length of the testes. Each

testis consists of lobes (Text-Fig. 6), each of which are again made up of several lobules called the seminiferous lobules. All the lobules have access to the *seminiferous duct* either directly or through others. The seminiferous ducts vary from 0·15 to 0·4 mm. in diameter and are lined with columnar epithelial cells. These cells are 40 to 45 micra in length. Nuclei of these cells are $3 \times 3{\cdot}5$ to $4 \times 4{\cdot}5$ micra. As described by Cronin (1947) in *Callinectes sapidus,* the wall of the lobules consists of two layers with characteristic nuclei without cell membranes. Each lobule consits of spermatocytes and accessory nuclei. The accessory nuclei are peripheral and without cell membranes. Most of them are round and slightly oval and are about $3{\cdot}1 \times 3{\cdot}5$ to 4×6 micra. All the spermatocytes of a single lobule are of the same stage of development. Young spermatocytes are $7{\cdot}5 \times 10{\cdot}5$ micra.

In some seminiferous lobules the wall gets modified to form a columnar epithelium with cells 23 to 25 micra long and 9 to 10 micra wide. Nuclei in these cells are median and round and $5 \times 5{\cdot}5$ to $6 \times 6{\cdot}5$ micra.

The *vas efferens* is a very small tube connecting the testis at the region behind the posterior corner of the cardiac foregut and the anterior coiled region of the vas deferens. It is embedded in the coiled anterior vas deferens so that it is difficult to be distinguished.

The *vasa deferentia* are a pair of very long convoluted tubes of varying width at different regions, the anterior, the median and the posterior vasa deferentia as described by Cronin (1947).

The *anterior vas deferens* (avd) is a very narrow transparent extremely convoluted tube forming a thick mass of tight coils. The whole mass rest on the thick anterior region of the median vas deferens. In a mature specimen the coils of the anterior vas are 0·5 mm. in width. The tube consists of a columnar epithelium (Text-Fig. 8) surrounded by a thin strata of muscle and connective tissues. The epithelial cells are 45 to 100 micra in length and 16 to 23 micra in width. The nuclei are median and round or oval.

The *median vas deferens* (mvd) is a very massive part and in mature crabs this portion of the two sides lie side by side behind the foregut, hiding the connection between the two testes. In mature specimens the median vas can be divided into two

regions an anterior thick portion consisting of large irregular coils formed as a single mass which is pink, and a posterior whitish region made up of slightly thinner white coils. Both the regions are full of white spermatophores and the tube at this region is 1·5 to 2 mm. in width. In younger specimens only one white coiled mass is present as median vas. The wall of the coils have the same structure as other portions of the vas deferens, an epithelial layer situated on a thin layer of connective tissue and muscles. Lateral pockets like those present in the posterior vas deferens are present on the walls of the median vas also.

The *posterior vas deferens* (pvd): Extends from the median vas to the base of the external penis on the ventral posterior side of the coxopodite. The posterior vas also can be divided into two regions, an anterior region consisting of transparent, thick, loosely convoluted and wide tube and a posterior narrow region. The anterior portion starts from the posterior end of the median vas and is situated below the pericardium on both sides. The region is of the same width as the median vas. The wall is full of small lateral pouches (Text-Fig. 11) which projects out from the wall. These pouches can be seen with the naked eyes. The tube is lined with epithelium the cells of which are 55 to 65 micra in length. The lateral pouches are also lined by epithelium, continuous with that of the main lumen of the tube. These pouches have a glandular function. The epithelial cells are of a glandular nature. When the walls of the transparent region are punctured a thick transparent fluid can be seen oozing out. This liquid may be the secretion of the glandular pouches and the epithelial cells of the tube. At the posterior end of the pericardium the anterior portion of the posterior vas deferens dips downwards in front of the antero-lateral prolongation of the median plate and passes outwards and backwards, through the muscles of the coxa and basiischium of the last walking leg, below the eighth thoracic arthrophragmal plate, and leads to the base of the external penis. This is the posterior region of the posterior vas deferens. It is a narrow more or less straight tube lined by low epithelium and is called the *ductus ejaculatorius* (ej.d).

The external penis is slightly wider than the ductus ejaculatorius and it is of the same structure as described by Cronin (1947). In *Carcinus maenas* Spaulding (1942) has described the tip of the genital papilla to be bearing small backwardly pointing

spines which hold the papilla in its place when inserted in the pleopod. But in *Neptuns sanguinolentus* such spines are absent at the tip of the genital papilla. According to Spaulding in *Carcinus maenas* the genital papilla is inserted into the pleopod without being able to be removed by any amount of bending of the abdomen in all the specimens examined by him. In *Neptunus sanguinolentus*, however, in immature specimens the genital papilla is not inserted into the pleopod, whereas in all the mature males these papillae remain inserted in the pleopods. Pearson (1908) suggests that in *Cancer pagurus* the papilla is inserted into the pleopod only during copulation.

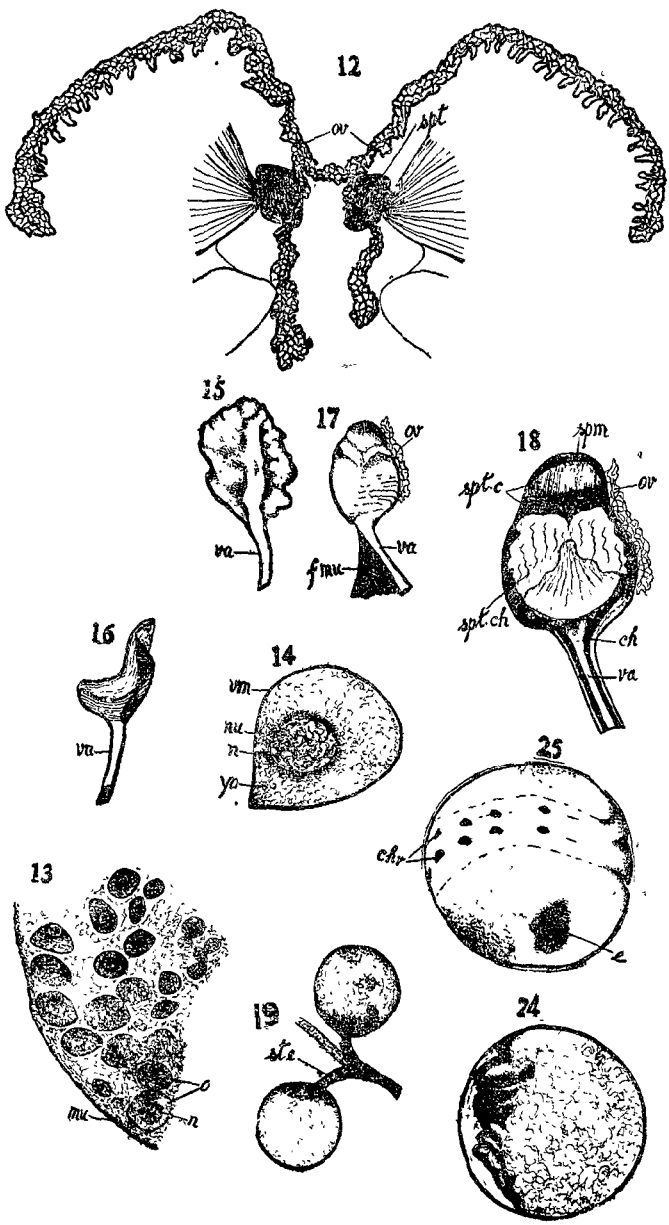
The mature spermatozoa (Text-Fig. 10) spheroidal structures having three or four rays. Both three and four rayed spermatozoa are produced as in *Cancer magister, Cancer productus* and *Cancer gracilis* (Fasten, 1918 & 1924) and *Carcinus maenas* (Spaulding 1942). In *Cancer oregonensis* only three rayed spermatozoa are present (Fasten, 1924). In *Menippe mercenaria* (Binford, 1913) the mature sperms are with five or more pseudopodial rays as in the crayfishes and lobsters. Each spermatozoon is about 4 micra in diameter. It measures 8·5 to 9·5 micra from the tip of one ray to that of another. The mature spermatozoa are packed in spermatophores. Unlike the spermatophores of the Anomura, which are pedunculated, those of *Neptunus sanguinolentus* are simple ellipsoidal capsules as those of *Carcinus maenas* (Spaulding, 1942). Most of them have a slight concavity on one side (Text-Fig. 9).

Female (Text-Fig. 12-18).

Females possess a broad abdomen and the dorsal surface of the carapace of mature females is more convex than that of males. Again in mature females the abdomen is not so closely applied to the thorax.

The female reproductive system (Text-Fig. 12) consists of a pair of ovaries, a pair of spermathecae, a pair of very short oviducts, and a pair of vagina from the posterior and of the spermatheca and opening to the exterior on the sixth thoracic sternum.

The ovaries are paired, tubular and lobulated organs occupying the same position as the testes of male. In ripe females the ovaries are orange red in colour and very thickly lobulated. In young ones they are only yellowish in colour and narrow. The



TEXT-FIGS. 12-19, 24, 25.

ovaries of the two sides are connected by a cross bar behind the foregut, in front of the heart and above the midgut. Just behind this transverse connection each ovary is connected by a very short *oviduct* with a spermatheca which in turn communicates with the exterior by a vagina. The oviduct is very short and covered by gonadial tissue. It runs for a short distance along the anterior inner side of the spermatheca and opens into it at about the middle portion. Due to the covering of the oviduct with the lobes of the ovary it is not visible to the outside and can be noted when dissected or in sections. Each ovary is prolonged backwards from the inner side of the spermatheca and this portion occupies a similar position as the posterior vas deferens of the male. Unlike that of *Cancer pagurus* the posterior prolongation of the ovary on the right side is shorter and narrower than that of the left side and it extends upto the middle of the inner curved edge of the eighth thoracic arthrophragm. On the left side it extends further posteriorly for about 5 or 6 mm. more. In *Cancer* Pearson has described these two posterior prolongations meeting together at the posterior and in mature specimen. But in *Neptunus sanguinolentus* this is not the case, the posterior extremities of the two ovaries remaining separate throughout the life of the crab.

In a transverse section through an ovary (Text-Fig. 13) can be seen numerous ova in various stages of development, the immature smaller ova situated near the centre portion and the bigger more mature ones towards the periphery. A mature ovum (Text-Fig. 14) is oval or round with a larger nucleus and a cytoplasm full of yolk granules, covered by a vitelline membrane.

The ova are situated in connective tissue containing scattered small cells, the nutritive cells. The ovary is covered by a thin outer membrane made up of connective tissue, muscles and epithelial lining cells.

The spermatheca or seminal receptacle is a sac-like organ into which opens the ovary by means of the short oviduct just behind the cross bar connecting the two sides of the ovary, situated just inside the inner corner of the fused thoracic epimera in a vertical position and occupying the same position as the anterior portion of the median vas deferens of the male. The size of the spermatheca varies considerably according to the condition of the animal. In young females the spermathecae are very small elongated sacs. In mature specimens they are large flat sacs with thick walls

(Text-Fig. 15) lying very close together due to their size and occupying the space between the heart and the pyloric foregut. In a soft shelled female which has recently copulated, the spermatheca is a large oval chamber with the upper one-fourth portion slightly marked off by a slight groove (Text-Fig. 17) and it contains the sperm plug.

The spermatheca consists of two parts, a glandular dorsal portion and a ventral portion lined with chitin from the base of which starts the vagina. The dorsal portion is lined by a very thick epithelium which is thrown into prominent ridges on the inside. The lower portion is lined with chitin which is continuous with the chitin of the vagina. The chitinous lining of the spermatheca and the vagina form a funnel-shaped structure with the expanded funnel region, which is ridged and wavy, placed in the lower half of the spermatheca. By pulling at the end of the vagina the whole of the chitinous funnel-shaped structure (Text-Fig. 16) can be pulled out. One side of the funnel is longer than the other side.

The *vagina* (oviduct of Pearson) is a wide duct lined on the inside with chitin continuous with the chitinous lining of the lower half of the spermatheca. It opens to the exterior at the middle of the sternum of the sixth thoracic somite. The outer posterior corner of the spermatheca and the outer side of the vagina are attached to the sternum by means of a characteristic fan-shaped muscle (Text-Fig. 17).

The *sperm plug* is a structure found inside the spermatheca of soft females which have recently copulated as described by Spaulding (1942) in *Carcinus maenas*. It is divided into two unequal portions, the upper part being only less than half the size of the lower portion. The place where the upper portion is marked from the lower can be noted even from the outside by the slight groove mentioned above. The upper portion fits in the anterior one-fourth region of the spermatheca with a thin epithelial layer covering it. In between the epithelial lining and the outer surface of this portion of the sperm plug are seen embedded some spermatophores and sperms. In formalin preserved specimens this portion solidifies and becomes very thick and rough, more or less like the thickly cuticularised portions of the body, yellowish brown in colour. This region itself can be divided into two portions, the lower one-fourth portion being of a more intense colour

than the upper portion (Text-Fig. 18). The line separating the two regions is prolonged downwards for a short distance at the centre as shown in the figure. The lower portion of the sperm plug occupies about three-fourth portion of the interior of the spermatheca. In formalin preserved specimens this portion remains as a jelly like substance in which are embedded some spermatophores. According to Pearson (1908) free spermatozoa are found inside the spermatheca. In *Neptunus sanguinolentus* spermatophores are found. Binford (1913) and Spaulding (1942) have also found spermatophores inside the sperm plug. In a longitudinal section dividing the whole spermatheca into two equal portions the prominent ridges on the inside of the epithelial lining of the lower portion of the wall of the spermatheca and the wavy lining of the chitin can be seen clearly through the jelly like substance. According to Spaulding the upper part of the plug is composed of a substance similar to the body cuticle and the lower part made up of chitin.

Embryological studies

The embryonic development of Brachyura does not appear to have received much attention. In the hermit crabs Mayer (1877) gave the first account of the embryology of *Eupagurus pridauxi*. Later Krainska (1936) worked out segmentation, gastrulation, etc. of the same species. In the Macrura the best known and one of the earliest work on embryology is that of Reichenbach (1886) on *Astacus*. The embryology of the American lobster was worked out by Bumpus (1891). Terao (1929) and Von Bonde (1936) have studied the embryology of the spiny lobster *Panulirus* and the Cape crawfish *Jassus lalandi* respectively. The embryology of *Caridina laevis* and *Palaemon idae* respectively were the subjects of the study of Nair (1949) and Iyer (1949).

The *fertilized egg* (Text-Fig. 19). The developing eggs are found attached to the setae on the pleopods of the female and they are carried on the ventral side of the abdomen. Each egg is covered by two membranes as observed by many authors on Crustacean eggs. According to Yonge (1937 & 1946) the outer membrane is cuticular and the inner chitinous.

It has not been possible to get any indication of the time of fertilization. Hence all reckoning of time or the age of the eggs here described is calculated backward from the time of hatching.

Egg three days before hatching (Text-Fig. 25): The egg is globular and yellowish red in colour due to the yolk granules, when viewed through the two transparent membranes. It measures 250 micra in diameter.

The first and second antennae, the mandible, the maxillae and the maxillipeds are developed as protruberances. The abdomen also is differentiated for a length of about 220 micra. The pigmentation of the eye is slightly visible on the two sides. In a section the eye is found to be an elongated structure which is slightly two-lobed, having a highest length and breadth of 75 and 35 micra respectively.

Egg two days before hatching (Text-Fig. 26): The appendages are elongated further. The eye has the same dimensions, but the pigmentation is more visible. Some of the nuclei get themselves arranged in a linear direction at the periphery.

In a transverse section both proctodaeum and stomodaeum are visible. The stomodaeum is 29×23 micra with cells 6 to 8 micra in length. The proctodaeum is 16×18 micra with cells 5 to 7 micra long.

The abdomen is more differentiated and is 132 micra wide. The telson has become definite in outline and is 47 micra in length.

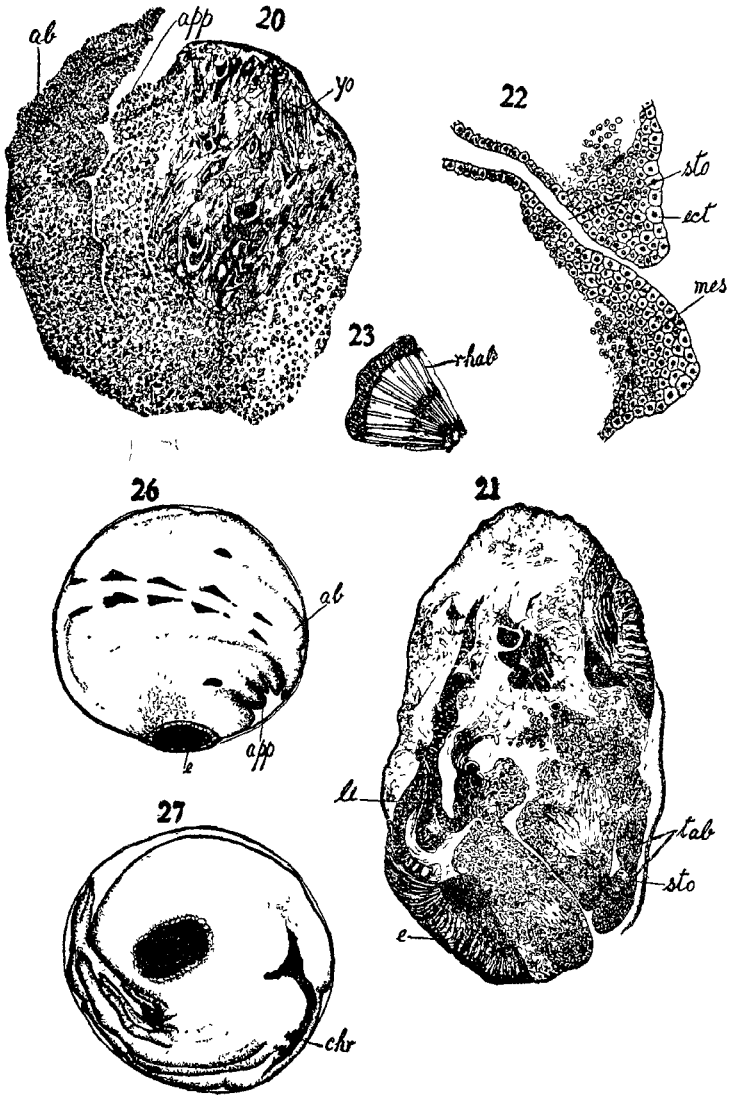
Egg, one day before hatching (Text-Fig. 27): The egg is about 290 micra in diameter. It has become black in colour due to the pigmentation of the eyes and the chromatophores. The yolk is much reduced and more or less transparent.

In this stage the embryo is fully developed and ready to hatch out. All the appendages of the larva are well developed. The abdomen is five segmented, the last segment bearing the telson.

The eye is also developed. Under double staining by heidenhain's iron haematoxylin and eosin the eye gets stained by eosin while the rest gets stained by haemotoxylin. The rhabdomes are developed as long parallel diverging rays towards the outside. In some the inner pigmented layer at the thickened portions of the rhabdomes as in the adults is also seen (Text-Fig. 23).

In a transverse section passing through the plane of the stomodaeum (Text-Fig. 21) the two eyes can be seen on the sides. In

between them on one side the cut end of the tip of the abdomen can be recognised. Inside that portion of the stomodaeum could be seen leading inwards. The ectoderm cells of the stomodaeum



TEXT-FIGS. 20-23, 26, 27.

(Text-Fig. 22) measures $3 \times 4 \cdot 5$ micra. Mesoderm cells are also more or less of the same dimensions, but slightly smaller towards the inside. Secondary mesoderm cells are smaller, measuring $1 \cdot 5 \times 2 \cdot 3$ to 3 micra.

The liver consists of lobes lined by epithelial cell measuring 9 to 12 micra in length. Yolk globules which get absorbed in the liver get stained dark in cross section.

Most of the chromatophores present in the larva are also visible in the egg at this stage.

An egg just before hatching measures 300 to 310 micra in diameter as against 360 to 375 micra observed in *Neptunus pelagicus* by Prasad and Tampi (1953). The fully developed embryo lies coiled inside the egg membranes with the telson extending in between the region of the two eyes. The appendages have the same dimensions as in prezoea. Tips of setae can be seen. During hatching the telson is the first region to come out and tips of the maxillipeds also project out. This is followed by the abdomen. After this with the help of the telson and the abdomen the anterior region also comes out of the egg case. The prezoea is covered by the embryonic cuticle when it comes out of the egg. The early and late first zoea stages of the species have been described by Raja Bai (1955).

EXPLANATION OF TEXT FIGURES

ab—Abdomen; ap—Aperture on the posterior face of the protopodite of the first pleopod of the male; app—Appendage; avd—Anterior vas deferens; ch—Chitin lining vagina and spermatheca; chr—Chromatophores; ct—Connective tissue; e—Eye; ect—Ectoderm; ejd—Ejaculatory duct, en—Endopodite; ep—Epithelial cells; ep gl l—Glandular epithelial pockets in the wall of the posterior vas deferens; ep sd—Epithelium of the seminiferous duct; fmu—Fan shaped muscle of the spermatheca; lb—Lobes of the testis; lbl—Lobules of the testis; li—Liver; mes—Mesoderm; mu—Muscle, mvd—Median vas deferens; n—Nucleus; nu—Nucleolus; o—Ova, ov—Ovary; pe—External penis; pr—Protopodites; pvd—Posterior vas deferens; rhab—Rhabdome; sd—Seminiferous duct; spm—Spermatophores inside the epithelial wall of the spermatheca; spt—Spermatheca; spt c—upper part of the sperm plug formed of cuticle; spt ch—Lower part of the sperm plug formed of chitin; st e—Stalk of the egg; sto—Stomodaeum; t ab—Tip of the abdomen; te—Testes; va—Vagina; vd—Vasa deferentia; vm—Vitelline membrane; yo—Yolk

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Stegodyphus Tibialis (Cambridge) (Family Eresidae: Araneida) from Madras

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ABSTRACT

In this paper a full description of the cribellate spider *Stegodyphus tibialis* (family Eresidae) is given as earlier descriptions are incomplete being based on a single male. Notes on nest and bionomics of the spider are also added and features peculiar to the species emphasised.

Introduction

Cambridge (1869) established this species by examining a single male specimen sent to him from Mysore (S. India). He described it as *Eresus tibialis*. This is adapted by Pockock (1900) who placed it under the genus *Stegodyphus* as *S. tibialis*. Thorell (1895) records of its occurrence in Burma without adding to the original description.

The first description of the species based on the male is incomplete in many ways as the palpal organ is not examined and figured. Subsequent to the original description neither the female nor the nest has been described. Since all other Indian species of *Stegodyphus* are distinguished chiefly by the characters of the female a full description of the female is given here for the first time. The present paper thus includes:

1. A detailed description of the female of *S. tibialis*.

2. The male is redescribed giving more details of measurements and the palpal organ is figured.

3. Notes on feeding habits and nest building.

4. The structure of the solitary nest with its mechanism for closing the entrance is described fully and compared with the nests of other species of the same genus.

Description: *Female* (*Plate* 1 *B*).

Stout built spiders clothered with golden yellow hairs on carapace and abdomen. The grey colour of the carapace can be seen under the pubescene of golden yellow hairs; but the abdomen is oval and uniformly golden yellow. Legs olive brown in colour and tipped black.

Cephalothorax, rectangular—length 7 mm. width 5 mm. The cephalic region high and thoracis region low. (Text-Fig. 1).

Eyes. 8. The anterior medians (AM) and posterior median (PM) forming an ocular quadrangle. The posterior laterals (PL) are situated far behind on the summit of the raised cephalic region. The median ocular quadrangle and the anterior lateral (AL) face forwards. The ratio of their diameter is AM : PM : AL : PL = 2 : 3 : 2 : 2, and their mutual distance AM-8. PM: 3·5. AM-PM = 2. AL-AM = 27·5. AL-PL = 10. PL-AM = 12. PL-PM = 10.

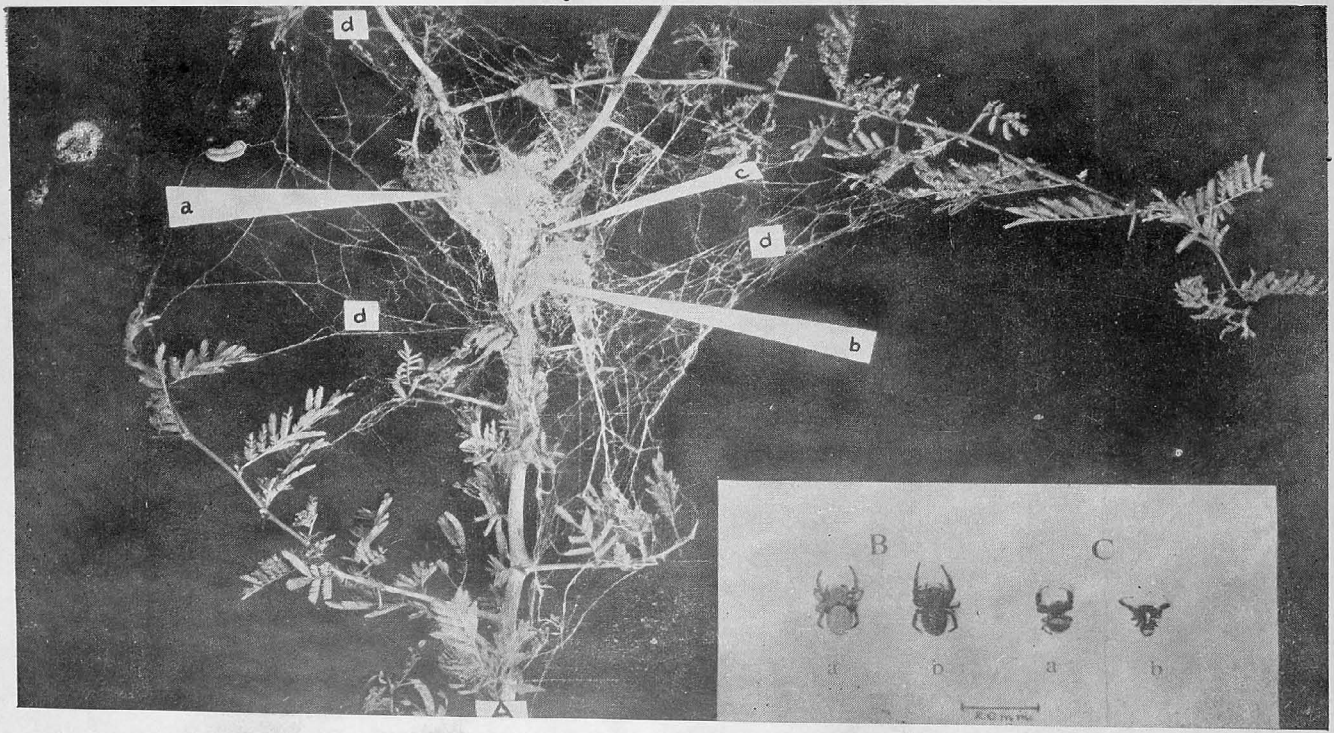
Clypeus. Narrow with a median and lateral projection with an inner curve connecting them (Text-Fig. 2).

Chelicerae. Length = 22 × width, black, flattened in front with yellow hairs on upper half. Fang short closing in an indistinct groove. A large tooth supported by short one on either side of its base seen. (Text-Fig. 3).

Labium. Long and setose (Text-Fig. 4) *Sternum* Ovate. *Maxillae* in the form of a broad lobe setose on the inner margin and with serrulae on outer margin, inside of maxillae with a pale yellow patch. Palp = 4·5 mm. Tarus of palps provide with many black spines and ending in a single claw. (Text-Fig. 4).

Legs. Upper side olive brown faintly banded and black at the tips. Measurements I—17. II—13·5, III—12, IV—15 mm. Metatarus of 4th leg bears the calamistrum (Text-Fig. 7).

Abdomen. Length 10 mm., width 7 mm. distinctly oval and golden yellow in colour. Upper side with 3 pairs of distinct pits of which the anterior ones are the most prominent. A faint 4th pair and transverse striae are also visible on closed observation. Underside of abdomen darker.



Stegodyphus tibialis (Cambridge) from Madras.

A. Nest: a—house, b—Tubular retreat, c—curtain, d—Net. B. Female: C. Male: a—Dorsal, b—Ventral views.

Epigyne. U shaped as in Text-Fig. 5.

Spinnerets:

Cribellum in the form of a broad plate bilobed and 3 pairs of spinnerets of which the inferior spinnerets are the largest (Text-Fig. 6). Total length. Female = 15 mm.

Description: Male, Plate I-C.

Cambridge emphasised the following features seen in the male.

1. First pair of legs with distinct enlargement of tibia provided with a close set of hairs.

2. Body black except for the yellow bands of hair on the lateral margin of carapace, a ventral band on the sternum, a dorsal band on abdomen, a circular patch encircling tip of abdomen and an oval yellow patch in front of spinnerets.

However the following features also show prominently when several spiders were examined.

1. First pair of legs very long and held outstretched like feelers during locomotion. Metatarsus with short tubercles beneath and tip of tarsus enlarged. (Text-Fig. 8).

2. All legs olive brown with a reddish tinge particularly the femur and patella of the first legs. The femur of the II, III and IV and the tibia of the first legs being the darkest. Metatarsus and tarsus of legs II, III and IV spined beneath.

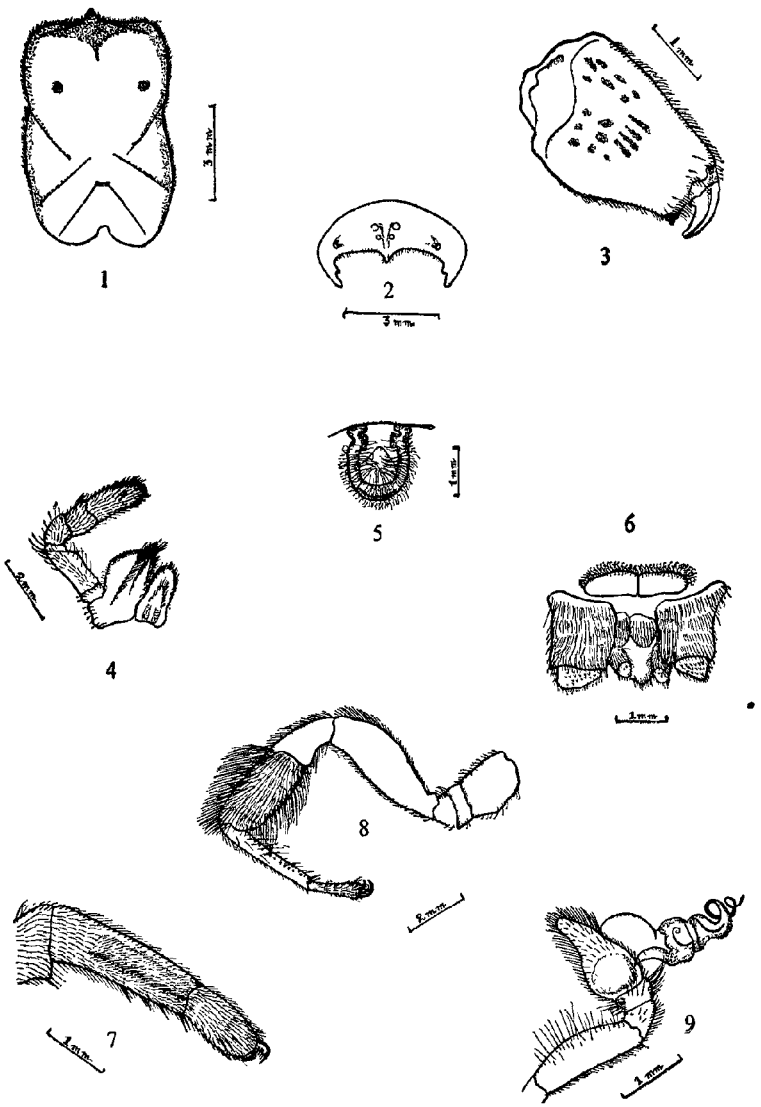
3. Papal organ complicated with a cymbium and style as in Text-Fig. 9. Cambridge does not describe this, "as the palpal organs were too much concealed in the dry specimen for satisfactory observation."

4. Measurements. Total lengths 9·2 mm. as against 8 mm. of Cambridge, and the cephalothorax and abdomen of almost same dimensions, Legs, I-14, II-8·5, III-7, IV-9 mm. Palp 3·2 mm.

5. In the male the Cribellum and the calamistum is poorly developed. They do not spin separate webs for themselves but live in a side chamber of the nest of the female.

Locality. 32 females and 9 males were collected from the scrub jungles of Tambaram. (8-9-1962).

Bionomics :



1 to 7—Female. 1. Carapace—dorsal view. 2. Carapace—Front view. 3. Chelicera. 4. Maxillae, labium and palp. 5. Epigyne. 6. Caribellum and spinnerets. 7. 4th leg—metatarsus showing *calamistrum*.

8 to 9—Male. 8. First leg. 9. Palpal organ.

Nests. Of about 35 nests examined 32 were found on thorny plants, like *Dichrostachys cineres* (Mimosoideae) *Zizyphus oenoplœs* (Rhamnaceae), *Randia dumatorum* (Rubiaceae) and *Scutia myrtina* (Rhamnacea) and 3 others on smooth stemmed plants like *Grewia hirsuta* (Tiliceae), *Cassia siamea* (Cesalphinodieae) which shows that they prefer thorny plants although smooth stemmed plants were found in equal numbers in the same locality. The female builds a solitary nest and lives inside unlike *S. sarsinorum* and *S. Socialis* found in the same locality that build massive nests and live together in numbers of 40 and more individuals. Unlike the nests of the related species the nest is kept clean, the dead bodies of smaller preys being dumped as refuse in one corner of the house. The nests are compact structures of irregular outline as shown in Plate IA. It measures roughly 60 cm. and consists of different parts. It consists of a wide strangling net extending in one or two direction to a distance of 50-60 cms. and is made of sticky threads designed to trap flying and jumping insects and other prey (d.) The tubular entrance is called the retreat and it opens into from below (b). This is 3-4 cms. long and leads into the compactly built house 5-6 cms. (a). At the entrance to the house is the "curtain" (c) formed of loose pad of soft yellow silk. This pad hangs by its upper margin on one side and when the entrance is disturbed it was noticed that the spider runs out of the house and draws down the pad of curtain with the front pair of legs. Since the pad is of sticky silk threads as the net, the pad is fastened to the lower margin of the entrance thus closing the burrow; with the result any intruder is kept out not only by the entrance being closed but by the sticky nature of the curtain. This curtain appears to be peculiar to the species and is not seen in the nest of *S. socialis* and S. *sarasinorum.*

Taxonomic Remarks. The definition of the species which has been established on the male type must be revised to incorporate the characters of the female since the latter are more abundant in the field. With the present description of the female the key given by Pockock for the identifications of the hitherto known in Indian species of the genus *Stegodyphus* can be revised as follows:—

Key to the identification of Females

A. Upper side of abdomen golden yellow.

a. Carapace black with lateral border of grey hairs.

S. socialis.

b. Carapace grey but clothed with uniform yellow hairs.

S. tibialis.

B. Upper side of abdomen clouded or banded with black.

a. Carapace clothed with olive black hairs, abdomen and not banded. *S. mirandus.*

b. Carapace clothed with grey white hairs abdomen banded.

b1. About 20 mm. in length mandibles whitish .

S. pacificus.

b2. About 10 mm. in length mandibles darkened.

S. sarasinorum.

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Phycomycetes in Agricultural Soils with Special Reference to Pythiaceae

1. Techniques of isolation *

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Introduction

Among the various micro-organisms inhabiting the soil, Phycomycetous fungi occupy a predominant place. Pythiaceae with six genera, viz. *Pythium, Phytophthora, Pythiogeton, Trachysphaerea, Diasporangium* and *Zoophagus* (Bisby, 1954) is one of the most important groups of soil fungi.

A review of literature indicates that no detailed study of the syn- or autecology of the Pythiaceae in soil has been made. This appears to be primarily due to the inadequacy of the usual isolation techniques employed in soil microbiology.

It is well known that the dilution plate technique of Waksman (1916, 1922) modified by Brierley *et al* (1927) most widely employed in soil microbiological studies is highly selective and is primarily a bacteriological technique which yields predominantly Fungi Imperfecti, Mucorales and a few Ascomycetes. Pythiaceous fungi do not generally appear on such dilution plates though they are known to occur commonly in most agricultural soils. Most of the later modifications of the above techniques such as those of Jensen (1931), Tyner (1944), Dowson (1947) and Martin (1950) etc. have not improved its efficiency in isolating Pythiaceae from soil. Other techniques devised by Chesters (1940, 1948), Meredith (1940) and La Touche (1948) have not been useful in the isolation of Pythiaceae without interference by other fungi.

* Formed part of a thesis submitted by the senior author for the M.Sc. degree of the Madras University.

Warcup (1950, 1951) has described the soil plate method which he found to have certain advantages over the dilution or direct methods. Genera like *Pythium* and *Mortierella* and several others have been isolated by this technique. However, the frequency of isolation of *Pythia* was not high. Campbell (1951) described an interesting technique for isolating *Phytophthora cinnamomi* Rand. from infected soils. Holes were bored in apples and these holes were filled with infested soils. The fungus that penetrated into the tissues of the host was easily brought into pure culture. This technique, however, was useful only in the case of specific fungal pathogens.

Thornton (1952, 1956) employed the screened immersion plate technique for isolating fungi in mixed oakwood soil profiles. He isolated over 50 species of fungi but no Pythiaceous fungi were obtained by this technique.

Butler (1907) adopted the baiting technique for the isolation of *Pythium* species and some of the Chytridiales. The baits floated were hemp (*Cannabis sativa* L.) seed, *Abutilon* roots, flies, meal worms, ants, cress seedlings, potato slabs etc. In order to check the growth of bacteria that were inhibiting the growth of fungi, the water was acidified by adding citric acid. By adopting this technique he isolated 18 species of *Pythium*. The baiting technique was later adopted by several investigators, viz. Couch (1939), Sparrow (1943), and Nicholls (1956).

Angell (1954) reported that by sieving soil and making an aqueous suspension, greater proportion of *Pythium* species get concentrated in the coarser portion of the soil. By this method he could make reliable estimates of the density of *Pythium* population in soil samples.

Barton (1958) found that turnip seedlings were successful as soil baits for the isolation of *Pythium*. Seeds of turnip sown in pots containing soil were incubated at 25°C and 80 to 100 per cent relative humidity. *Pythium mamillatum* Meurs. present in these soils infected most of the seedlings which showed symptoms of 'damping off'. It is obvious however, that only pathogenic species can be isolated by this technique.

Recently an agar block technique for the isolation of Pythiaceous fungi was described by Rangaswamy (1958). This technique is reported to be useful specially for the isolation of Pythiaceous fungi.

From the foregoing brief review it is evident that none of the techniques so far available except the agar block technique and the baiting technique can be consistently adopted to isolate Pythiaceous fungi from the soil. The potentialities of these two techniques have not also been fully exploited. Primarily because of the inadequacy of techniques our knowledge of the ecology of the Pythiaceae in soils is meagre and there are only a few references in literature bearing on this subject (Roth and Riker, 1943; Buchholtz, 1938; Remy, 1949; Chesters, 1949; Warcup, 1952; Takahashi, 1952; Stenton, 1953 and Barton, 1958). The present investigation was taken up to study the Phycomycetes of agricultural soils with special reference to Pythiaceae. In the course of the study the suitability of some of the techniques for isolating Phycomycetes was evaluated.

Material and methods

Materials

Soil samples: Four different types of soils from the farms attached to the Agricultural College and Research Institute, Coimbatore were taken up for investigation. These were

1. Dry land soil grown to Millets and Pulses, entirely rainfed. (Soil-A).

2. Garden land soil grown to vegetables and sweet potatoes under irrigation (Soil-B).

3. Wet land soil grown to rice (Soil-C).

4. Semi-wet land soil grown to bananas under irrigation (Soil-D).

The soil samples were taken with sterilized instruments from the upper six inches of the soil profile after scraping off the top soil. The samples were collected at random from different places in each field and a composite sample of each type made by mixing thoroughly. Sampling of the above four different types of soil was done during the first week of every month and repeated for a period of twelve months. The soil samples were simultaneously utilized for isolating fungi belonging to the Pythiaceae and other aquatic Phycomycetes by adopting different isolation techniques.

Methods

(a) *Techniques of isolation of fungi*

(i) *Baiting technique* (Butler, 1907): Crystallizing dishes of 250 ml capacity and measuring 9.5 cm. in diameter and 4 cm. in height were covered with lids. The dishes were filled with 250 ml of distilled water and sterilized in an autoclave at 20 lb. pressure for 30 minutes. The sterilized dishes were placed on a table in the laboratory and 10 gm. of the representative samples of the different soils were gently distributed at the bottom of these dishes (Plate 1—Fig. 1). The floating material on the surface of the water in the dish was carefully removed with sterilised filter paper. The filter or blotting papers were sterilized in a hot air oven for two hours.

Three different types of baiting materials, viz. (1) cellophane paper bits (2) bits of cockroach (*Periplaneta americana* L.) wing and (3) dead ants (*Solenopsis geminata* E.) were used in the study. Cellophane paper and cockroach wings were cut into very small bits (approximately 0·2″ squares) while the ants were used as such. These baits were placed in test tubes in sterile water and steam sterilized for 15 minutes and transferred separately to the surface of the water in each crystallizing dish aseptically. Ten bits were allowed to float on the surface of the water in each dish. Care was taken to avoid disturbing the dishes after the baits were suspended since frequent disturbance of the dishes caused spores of Mucorales and Fungi Imperfecti to come up and colonise the floating baits. This interfered with the observation of the aquatic Phycomycetes colonising the baits. Within 5-7 days the baits were examined and the fungi were isolated to slants. The baits were found constantly contaminated with bacteria which inhibited the growth or arrested the development of fungi. Bacterial contamination on the baits was overcome by the addition of streptomycin sulphate at 1000 p.p.m. to the water in the crystallizing dishes.

(ii) *Agar block method* (Rangaswamy, 1958): Glass rods of 0·2″—0·3″ in thickness were bent into rectangular moulds measuring 3″ in length and 1″ in breadth and these moulds were kept inside a pair of petri-dishes as shown in Plate 2—Fig. 1 and sterilized in the hot air oven at 160°C for two hours. After sterilization these were allowed to cool and melted oat agar at about 40°C was poured into the mould to fill the volume as shown in Plate 2—Fig. 2. The agar blocks were gently removed from the dishes and buried in each type of soil in the different fields during

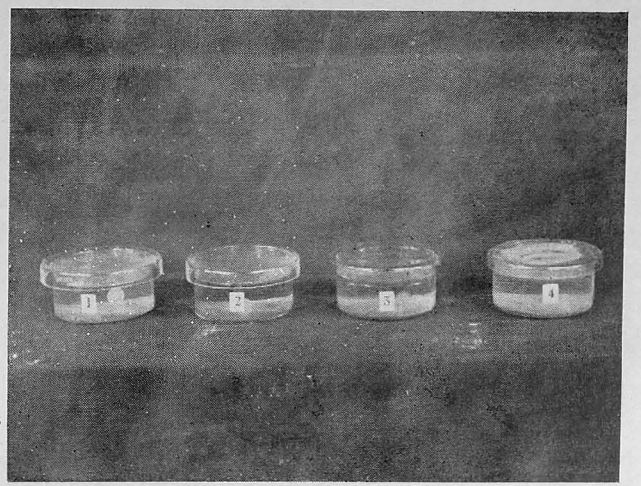


FIG. 1. Baiting technique showing the arrangement of the dishes with soil samples, water and baits.

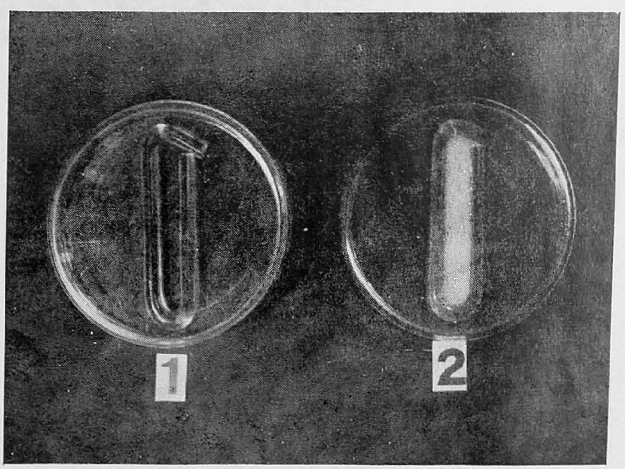


FIG. 2. Agar block technique.

1. Glass rod ready for pouring the agar. 2. Glass rod mould with the agar poured and set, ready for burying in the soil.

the first week of every month. Small pits were dug to a depth of 3", and the agar blocks placed inside the pits and the soil replaced. Agar blocks were buried at the rate of four in each field representing the four different types of soils and these were allowed to remain buried in the different fields for 6, 12, 24 and 48 hours. After the specified incubation period, the agar blocks were carefully removed by lifting the blocks with a portion of the soil all round it by means of a sterilized shovel. By means of a sterilized scalpel and forceps the soil round the blocks were carefully removed without any disturbance to the medium in the mould and transferred to sterilized petri-dishes. These were brought to the laboratory and the soil particles adhering to the surface of the agar blocks as well as portions of the agar medium were carefully scraped off by means of a sterilized scalpel and small bits of agar were cut under aseptic conditions. Six bits were cut from each block and these bits were plated in six petri-dishes containing oat agar medium. The resulting mycelial growth from the agar blocks plated was examined and the various isolates of Pythiaceous fungi from different types of soils were studied.

(iii) *Dilution plate method:* Two dilutions, viz. 1:50,000 and 1:100,000 were used in the studies. This was done by progressive dilution (Waksman, 1922). There were three replications for each dilution and from each type of soil.

Experimental results

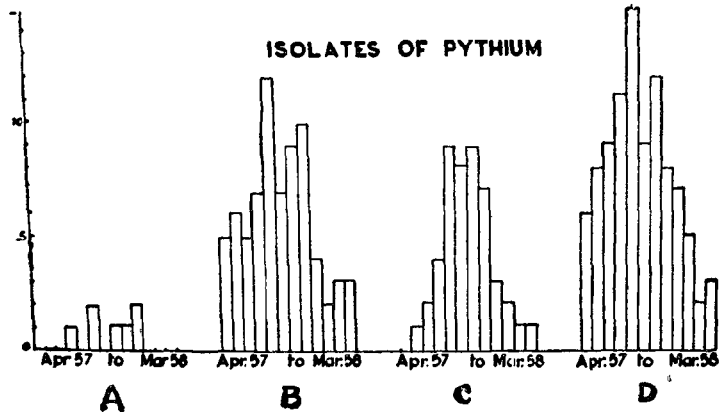
Techniques: The three techniques, viz. baiting technique (Butler, 1907), agar block technique (Rangaswamy, 1958) and the dilution plate technique (Waksman, 1922) were adopted for the isolation of Pythiaceae and other Phycomycetes from all the four soils, viz. dry land soil, garden land soil, wet land soil and semi-wet land soil. Three different baits, viz. cockroach wing, cellophane and ant were employed in the studies. The results obtained in the three techniques are detailed below.

(i) *Baiting technique.* In this experiment it was sought to acertain the following:

(1) To assess the suitability of the technique for ascertaining the relative abundance of Pythiaceae in the four soils studied; incidentally, the occurrence of Chytridiales has also been taken into account.

(2) To find out the relative efficiency of the baits employed in the study for the isolation of Pythiaceae, and

(3) Whether the efficacy of the baits was in any way modified by the soils used.



TEXT-FIG. 1. Comparison of the number of isolates of *Pythia* from the four different soils. A. Dry land soil. B. Garden land soil. C. Wet land soil. D. Semi-wet land soil.

(4) Whether the relative efficacy of the baits varied in the different months of the experiment.

The numbers of *Pythia* and Chytridiaceous fungi isolated from the four soils during April 1957 to March 1958 using three baits are given in table 1.

TABLE 1

Number of Pythia and Chytridiales obtained from the four soils during a period of twelve months using three baits

Soils	BAITS					
	Cockroach		Cellophane		Ant	
	Pythium	Chytridiales	Pythium	Chytridiales	Pythium	Chytridiales
Dry land soil	8	54	Nil	35	2	21
Wet land soil	38	97	2	31	28	46
Garden land soil	60	114	3	48	37	32
Semi-wet land soil	78	128	3	67	49	47
Total	184	393	8	181	116	146

The data were statistically analysed. The analysis of variance is furnished in table 2.

TABLE 2

Pythium and Chytridiales obtained from the four soils during twelve months using three baits and two bactericides

Analysis of variance

S. No.	Source of variation	D.F.	S.S.	M.S.	"F" value
1.	Baits	2	32·96	16·48	59·71 **
2.	Soils	2	3·86	1·93	6·99 **
3.	Bactericides	2	30·97	15·485	56·11 **
4.	Months	11	35·95	3·27	11·85 **
5.	Fungi	1	14·76	14·76	53·48 **
6.	Baits × Soils	4	0·58	0·145	—
7.	Baits × Bactericides	4	2·56	0·64	2·32
8.	Baits × Months	22	8·68	0·394	1·43
9.	Baits × Fungi	2	6·37	3·185	11·54 **
10.	Soils × Bactericides	4	0·11	0·027	—
11.	Soils × Months	22	7·64	0·347	1·26
12.	Soils × Fungi	4	0·32	0·115	—
13.	Bactericides × Months	22	7·72	0·35	1·27
14.	Bactericides × Fungi	2	1·73	0·865	3·13 *
15.	Months × Fungi	11	6·99	0·635	2·30 **
16.	Baits × Soils × Bactericides	8	1·05	0·131	—
17.	Baits × Soils × Months	44	14·73	0·334	1·21
18.	Baits × Soils × Fungi	4	1·09	0·272	—
19.	Baits × Bactericides × Months	44	9·62	0·218	—
20.	Baits × Bactericides × Fungi	4	5·43	1·357	4·92 **
21.	Baits × Months × Fungi	22	17·54	0·797	2·89 **
22.	Soils × Bactericides × Months	44	6·96	0·158	—
23.	Soils × Bactericides × Fungi	4	0·68	0·17	—

S. No.	Source of variation	D.F.	S.S.	M.S.	"F" value
24.	Soils × Months × Fungi	22	5·46	0·248	—
25.	Bactericides × Months × Fungi	22	7·14	0·324	1·17
26.	Baits × Soils × Bactericides × Months	88	35·14	0·399*	
27.	Baits × Soils × Bactericides × Fungi	8	0·44	0·055	
28.	Baits × Bactericides × Fungi × Months	44	7·64	0·173	0·276
29.	Baits × Soils × Fungi × Months	44	2·96	0·067	
30.	Soils × Bactericides × Months × Fungi	44	5·73	0·13*	
31.	Baits × Soils × Bactericides × Months × Fungi	88	7·13	0·081	
	Total	647	289·85		

** Significant at 1 per cent level.

* Significant at 5 per cent level.

The results indicate that the kind of baits used as well as the other factors studied have exerted a marked influence on the number of isolates. The summary of results and conclusions arising therefrom are given in tables 2a, b, c, d and e.

*Note: In the analysis of the data, the dry land soil was deleted in view of the fact that very few isolates were obtained from this soil, there being no isolates in most of the months. Due to the presence of some zeros in the data of the other three soils, the figures were suitably transformed before analysis to justify the assumption of normal distributon.

The five factor interaction (No. 31) was assumed to represent the experimental error and used to test all the other components. The high significance obtained in respect of almost all the components suggested that the error used for testing might be an under estimate of true error. Therefore the next lower order, viz. the four factor interactions (non-significant) Nos. 26, 28 and 30 were pooled together and used for testing the main effects and the two factor and three factor interactions The 'F' values obtained in this test are furnished in the table.

1. *Suitability of the technique for ascertaining the relative abundance of Pythiaceae and Chytridiales in the four soils.*

TABLE 2-(a) *Number of Pythia and Chytridiales Comparison of fungi*
(Summary of results)

S. No.	Baits	Mean number of isolates (Transformed)	S.E.	C.D. (P=0·05)
1.	Pythium	1·05	0·0292	0·081
2.	Chytridiales	1·36		

Conclusion: 2, 1

The results indicate (tables 1 and 2) that there are differences in the numbers of *Pythia* isolated from the four soils. These differences are statistically significant. It is therefore concluded that the baiting technique is a suitable one for ascertaining the relative abundance of *Pythia* whether cockroach or ant is used as the bait. The same trend of differences in numbers of isolates between soils is noticed in the case of Chytridiaceae also. The differences were statistically significant. The baiting technique is therefore suitable for demonstrating the relative abundance of this group of fungi also.

It is observed that Chytridiales occur in significantly greater numbers than *Pythia* when the three soils are considered. The position is the same when the soils are considered individually. It is likely that Chytridiales are more prolific in the soils studied than *Pythia* or the baiting technique is relatively more efficient in isolating Chytridiales than *Pythia*.

2. *Relative efficacy of the baits for the isolation of Pythia and Chytridiales.*

TABLE 2-(b) *Comparison of Baits*
(Summary of results)

S. No.	Fungi	Mean number of isolates (Transformed)	S.E.	C.D. (P=0·05)
1.	Cockroach wing	1·51		
2.	Cellophane	0·98	0·0358	0·099
3.	Ant	1·13		

Conclusion: 1, 3, 2

A comparison of the three baits indicates that cockroach wing bait has recorded the maximum number of isolates which is significantly greater than cellophane and ant.

TABLE 2-(c)

Interaction effects (Baits × Fungi)

(Summary of results)

S. No.	Baits	Fungi Mean number of isolates (Transformed) Pythium	Chytridiales	S.E.	C.D. (P=0·05)
1.	Cockroach	1·30	1·72		
2.	Cellophane	0·75	1·21	0·0505	0·14
3.	Ant	1·11	1·14		

Conclusions:

(a) Pythium — Cockroach, Ant, Cellophane.
Chytridiales — Cockroach, Cellophane, Ant.

(b) Cockroach — Chytridiales, Pythium.
Cellophane — Chytridiales, Pythium.
Ant — Chytridiales, Pythium.

The interaction between baits and fungi presented in table 2(c) also shows that cockroach wing as a bait is significantly superior to ant and cellophane in isolating both *Pythia* and Chytridiales. Chytridiales have been isolated in significantly greater numbers than *Pythia* by using cockroach and cellophane while by using ant as a bait the number of isolates of the two fungi are on a par.

3. *Efficacy of the baits in relation to soils.*

A reference to table 2 indicates that the interaction between baits and soils is not statistically significant. This shows that whatever be the type of soil, the relative efficacy of the three baits remains unaltered.

4. *Relative efficacy of the baits in relation to different periods of isolation.*

The interaction between baits and months as well as between baits, soils and months (vide table 2) are not statistically significant. However, the interaction between baits, fungi and months has attained the level of significance. The results are presented in table 2(d).

TABLE 2-(d)

Interaction effects (Baits × Fungi × Months)

(Summary of results)

S. No.	Months	Cockroach		Cellophone		Ant	
		(Mean No. of isolates transformed)					
		Pythium	Chytridiales	Pythium	Chytridiales	Pythium	Chytridiales
1.	April	1·44	0·99	0·71	0·86	0·82	0·71
2.	May	0·84	1·01	0·71	0·99	0·84	0·81
3.	June	1·53	1·55	0·71	1·05	0·89	0·87
4.	July	0·99	1·72	0·71	1·29	1·14	1·10
5.	August	1·78	1·94	0·82	1·36	1·43	1·16
6.	Sept.	1·47	1·88	0·77	1·40	1·16	1·23
7.	Oct.	1·72	1·93	0·71	1·32	1·42	1·21
8.	Nov.	1·51	2·50	0·88	1·48	1·71	1·19
9.	Dec.	1·53	2·29	0·82	1·68	1·46	1·97
10.	Jan.	1·16	1·49	0·71	0·92	0·82	1·71
11.	Feb.	0·81	1·85	0·71	0·86	0·81	0·84
12.	March	0·81	1·43	0·71	1·29	0·89	0·96

S.E.: 0·175 C.D. (P=0·05) 0·49

The results indicate that during July to December, the numbers of Chytridiales isolated are relatively greater both with cockroach and cellophane baits while in the case of ant, more of *Pythia* have been isolated (Table 2-d).

Table 2 (d) also indicates that cockroach and ant rarely differ in their efficacy in respect of isolating *Pythium* but in the isolation of Chytridiales, cockroach is largely the most efficient bait.

Table 2 (d) shows that in respect of both kinds of fungi the isolates are relatively more numerous during July to December, irrespective of the three baits used.

It is also observed from table 2 that the relative efficacy of the baits could be improved by the use of bactericides. Streptomycin sulphate was significantly more efficient as a bactericide in inhibiting the growth of bacteria. The interactions viz., bactericides × fungi and baits × bactericides × fungi were also found to be statistically significant.

(ii) *Agar block technique*:

This technique (vide "Materials and Methods") was adopted for the isolation of *Pythia* from the four different types of soil taken up for investigation. The total number of isolates of *Pythia* obtained from the four soils by adopting this technique, as compared to the baiting technique are presented in table 3.

TABLE 3

Number of isolates of Pythia obtained from the four soils by adopting the agar block and the baiting technique

S. No.	Soils	Agar block technique	Baiting technique		
			Cock roach	Cello-phane	Ant
1	Dry land soil	7	8	Nil	2
2	Wet land soil	47	38	2	28
3	Garden land soil	73	60	3	37
4	Semi-wet land soil	95	78	3	49
	Total	222	184	8	116

It is seen from the data that large number of *Pythia* have been isolated by adopting both the techniques. It is also evident that the agar block technique has given larger number of isolates of *Pythium* than any *one* of the baits used in the baiting technique.

The data obtained in the agar block technique are presented in table 4.

TABLE 4

Numbers of isolates of Pythia obtained from four different soils during April 1957 to March 1958

S. No.	Period	Dry land soil	Garden land soil	Wet land soil	Semi-wet land soil
1	April	—	5	—	6
2	May	—	6	1	8
3	June	1	5	2	9
4	July	—	7	4	11
5	August	2	12	9	15
6	September	—	7	8	9
7	October	1	9	9	12
8	November	1	10	7	8
9	December	2	4	3	7
10	January	—	2	2	5
11	February	—	3	1	2
12	March	—	3	1	3
	Total	7	73	47	95

The data were statistically analysed and the results are presented in table 4 (a).

Note: (The dry land soil has not been taken into consideration in the analysis of the data in view of the fact that it shows the least number of isolates, having recorded no isolates at all during seven months of the year. Hence it is obvious that this soil is clearly not congeniai for the occurrence of *Pythia*. The interaction between months and soils was taken as the error for comparing differences between months and between soils).

TABLE 4 (a) *Analysis of variance*

Source of variation	D.F.	S.S.	M.S.	'F' value
Months	11	330·97	30·09	13·31**
Soils	2	96·22	48·11	21·28**
Interaction	22	49·78	2·26	—
Total	35	476·97		

** Significant at the 1 per cent level.

Summary of results

(i) Comparison of soils.

S. No.	Soils	Mean number of isolates from each oil	S.E.	C.D. (P=0·05)
1	Garden land soil	6·08		
2	Wet land soil	3·92	0·43	1·26
3	Semi-wet land soil	7·92		

Conclusions: 3, 1, 2

The data show that the semi-wet land soil has recorded the maximum number of isolates of *Pythium* followed by garden land soil, the wet land soil recording the least number.

The results when compared to the relative number of isolates recorded in the different soils by using the baiting technique (table 6) show the same trend, except that in the baiting technique, the garden land and the wet land soil are on a par.

(ii) Comparison of months.

S. No.	Months	Mean isolates per month	S.E.	C.D. (P=0·05)
1.	April	3·67		
2.	May	5·00		
3.	June	5·33		
4.	July	7·33		
5.	August	12·00		
6.	September	8·00		
7.	October	10·00	0·87	2·55
8.	November	8·33		
9.	December	4·67		
10.	January	3·00		
11.	February	2·00		
12.	March	2·33		

Conclusions:

5, 7, 8, 6, 4, 3, 2; 9; 1; 10; 12; 11

The results indicate that approximately from July to November the number of isolates obtained was greater than during the rest of the year.

In the baiting technique also (table 6 (ii)) relatively greater number of isolates were obtained from July to December than during the other months.

Thus, the inference appears to be justified that in both the techniques the maximum number of isolates of *Pythium* was obtained during August to November irrespective of the techniques adopted. The reasons for the monthly fluctuation will be examined while discussing the effect of edaphic factors on the numbers of *Pythium* spp. elsewhere.

It also appears that both the techniques are quite similar as regards the relative abundance of the isolates of *Pythium* in the different soils and during the different months of the year.

Number of isolates of Pythiaceous fungi from agar blocks buried for four different durations.

As already described (vide "Materials and Methods") the agar blocks were buried in the different soils for a period of twelve months during the first week of every month and allowed to remain in the soil for 6, 12, 24 and 48 hours. The agar blocks were thereafter carefully removed from the soil and the fungi isolated. The data obtained are presented in table 5. The results of statistical analysis are furnished in table 5 (a), (i), (ii) and (iii.).

TABLE 5

Number of isolates of Pythium obtained from four different soils from agar blocks buried for four different durations during April 1957 to March 1958.

Period	Dry land				Garden land				Wet land				Semi-wet land			
	6	12	24	48	6	12	24	48	6	12	24	48	6	12	24	48
1957																
April				..		1	4							3	2	1
May					1	1	2	2			1			6	2	
June		1			2		1	2			1	1	1	3	1	4
July					1	1	2	3		1	2	1	2	2	4	3
August	2				1	4	3	4		3	3	3		6	6	3
September						2	4	1	1	2	3	2	1	2	3	3
October			1			4	4	1	2	2	4	1		3	6	3
November		1				1	5	4	2	2	1	2		1	2	5
December			1	1		3	1		1			2		3	1	3
1958																
January							2			1		1		1	4	
February						1	2				1				.2	
March						2	1			1					2	1
Total	1	3	2	1	5	20	31	17	6	12	16	13	4	30	35	26

Note: (In this analysis also the dry land soil has been deleted since it recorded no isolates at all during the seven months of the year. Due to the presence of some zeros in the data of the other three soils, the figures were suitably transformed before analysis to justify the assumption of normal distribution.)

TABLE 5 (a) *Analysis of variance*

Sources of variation	D.F.	S.S.	M.S.	'F' value
Months	11	10·1912	0·9265	6·08**
Soils	2	2·4797	1·2398	8·14**
Durations	3	8·9946	2·9982	19·67**
Months×Soils	22	1·9495	0·0883	—
Months×Durations	33	6·4374	0·1951	1·38
Soils×Durations	6	1·6701	0·2784	1·97
Months×Soils×Durations	66	9·3130	0·1411	
Total	143	41·0304		

** Significant at the 1 per cent level.

Summary of results

(i) Comparison of durations

S. No.	Durations	Mean isolates per month (transformed)	S.E.	C.D. (P=0·05)
1.	6 hours	0·91		
2.	12 „	1·39	0·0651	0·1804
3.	24 „	1·59		
4.	48 „	1·33		

Conclusion: 3, 2, 4, 1

The results regarding the number of isolates from different durations reveal that significantly greater number of isolates was obtained from agar blocks buried for 24 hours' duration than from other durations. The number of isolates from agar blocks buried for 12 hours and 48 hours were on a par while that from six hours recorded the least number of isolates.

(ii) Comparison of soils

S. No.	Soils	Mean number of isolates per month (transformed)	S. E.	C.D. (P=0·05)
1.	Garden land soil	1·32		
2.	Wet land soil	1·14	0·0563	0·1560
3.	Semi-wet land soil	1·46		

Conclusions: 3, 1, 2

It is seen that semi-wet land soil and garden land soil are on a par while the wet land soil has recorded significantly least number.

(iii) Comparison of months

S. No.	Months	Mean number of isolates per month (transformed)	S. E.	C.D. (P=0·05)
1.	April	1·08		
2.	May	1·21		
3.	June	1·28		
4.	July	1·48		
5.	August	1·78		
6.	September	1·54	0·1127	0·3123
7.	October	1·64		
8.	November	1·52		
9.	December	1·20		
10.	January	1·03		
11.	February	0·94		
12.	March	0·98		

Conclusions:

5, 7, 6, 8, 4, 3, 2, 9, 1, 10, 12, 11

The results indicate that approximately from July to November the number of isolates obtained was greater than during the rest of the year.

(iii) *Dilution plate technique*:

In this technique two dilutions, viz., 1:50,000 and 1:100,000 were employed for the isolation of Pythiaceous fungi. But no

isolates of *Pythium* could be obtained revealing thereby the unsuitability of this technique in the isolation of *Pythium* spp.

QUANTITATIVE AND QUALITATIVE DIFFERENCES AND SIMILARITIES IN PHYCOMYCETEOUS FLORA BETWEEN THE FOUR SOILS

At this stage it is of interest to refer to the results obtained in the baiting technique (table 2). In this case there are significant differences between the soil types in the number of isolates of *Pythium* and Chytridiales obtained during the different months of the year (tables 6(i) & (ii). The differences in the number of isolates recorded during the different months as well as the interaction between months and fungi are also significant (table 7).

TABLE 6

Baiting technique—Number of isolates of Pythium and Chytridiales obtained from the three soil types.

Summary of results of table 2

(i) Comparison of soils.

S. No.	Soils	Mean number of isolates (transformed)	S. E.	C.D. (P=0·05)
1.	Garden land soil	1·20		
2.	Wet land oil	1·11	0·0358	0·099
3.	Semi-wet land soil	1·30		

Conclusions: 3, $\overline{1, 2}$

It is seen that semi-wet land soil has recorded significantly more number of isolates than the other two soils which were on a par.

A comparison of the number of isolates from the four soil types by adopting the two techniques indicate that the maximum number of isolates are obtained from the semi-wet land soil followed by the garden land soil and dry land soil in both the techniques and the differences are found to be statistically significant (Table 4 a(i) and table 6(i).

(ii) Comparison of months.

S. No.	Months	Mean number of isolates (*transformed*)	S. E.	C.D. (P=0·05)
1.	April	0·92		
2.	May	0·87		
3.	June	1·10		
4.	July	1·16		
5.	August	1·41		
6.	September	1·32	0·0715	0·198
7.	October	1·39		
8.	November	1·55		
9.	December	1·60		
10.	January	1·15		
11.	February	0·98		
12.	March	1·01		

Conclusions:

$$\overline{9,\ 8,\ 5,\ 7,\ 6,\ 4,\ 10,}\quad \overline{3,\ 12,\ 11,}\ 1,\ 2$$

It may be seen from the general trend of results that the isolates are more in number during the period from July to December than during the other months.

The data from the two techniques during the different months reveals that maximum number of isolates of *Pythia* were obtained from August to November irrespective of the techniques adopted. In both the techniques the periods January to June were found to be not favourable for the isolation of *Pythia*. (Table 4 a (ii) and table 6 (ii).

The results obtained from the two techniques viz., baiting technique and the agar block technique are similar as regards the relative abundance of the isolates of *Pythium* irrespective of the soil types.

TABLE 7

Interaction effects (months × fungi)

(*Summary of results of table* 2)

S. No.	Months	Mean number of isolates (transformed)		S. E.	C. D. (P=0·05)
		Pythium	Chytridiales		
1.	April 1957	0·99	0·86		
2.	May	0·80	0·94		
3.	June	1·04	1·15		
4.	July	0·95	1·37		
5.	August	1·34	1·49		
6.	September	1·13	1·50	0·101	0·28
7.	October	1·30	1·49		
8.	November	1·37	1·73		
9.	December	1·27	1·94		
10.	January 1958	0·90	1·41		
11.	February	0·77	1·19		
12.	March	0·79	1·22		

Conclusions:

(i)

Months	*Fungi*
1. April	*Pythium, Chytridiales*
2. May	*Chytridiales, Pythium*
3. June	*Chytridiales, Pythium*
4. July	*Chytridiales, Pythium*
5. August	*Chytridiales, Pythium*
6. September	*Chytridiales, Pythium*
7. October	*Chytridiales, Pythium*
8. November	*Chytridiales, Pythium*
9. December	*Chytridiales, Pythium*
10. January	*Chytridiales, Pythium*
11. February	*Chytridiales, Pythium*
12. March	*Chytridiales, Pythium*

(ii) *Fungi*.

Pythium 8, 5, 7, 9, 6, 3, 1, 4, 10, 2, 12, 11

Chytridiales 9, 8, 6, 7, 5, 10, 4, 12, 11, 3, 2, 1

The interaction between months and fungi indicates the predominance of Chytridiales over *Pythium* spp. during the entire period except the month of April. However, the differences are significant during the months of July and November to March.

Both types of fungi are found to thrive better during the months of August to December.

A comparison of the species of *Pythium* obtained by using the two techniques reveals that larger variety of species are obtained by the agar block method than by the baiting technique (table 8). Agar block method is found to be less selective and permits wider number of species to be isolated.

TABLE 8

Comparative list of species of Pythium isolated by the agar block and the baiting technique

	Agar block technique.	*Baiting technique.*
1.	*Pythium aphanidermatum.*	1. *Pythium aphanidermatum.*
2.	„ *carolinianum.*	2. „ *carolinianum.*
3.	„ *spinosum.*	3. „ *spinosum.*
4.	„ *periplocum.*	4. „ *periplocum.*
5.	„ *indicum.*	5. „ *indicum.*
6.	„ *debaryanum.*	6. „ *debaryanum.*
7.	„ *graminicolum.*	
8.	„ *catenulatum.*	
9.	„ *proliferum*	
10.	„ *helicoides.*	
11.	„ *oedochilum.*	
12.	„ *lobatum**	
13.	„ *parasiticum**	
14.	„ *middletonii**	
15.	„ *drechsleri**	

* n. sp. to be described elsewhere.

Discussion

The present investigation was taken up with the objective of studying the distribution and behaviour of Phycomyceteous fungi in agricultural soils with special reference to Pythiaceae. Among the Phycomyceteous fungi causing destructive plant disease, *Pythium* spp. occupy a predominent place. The genus *Pythium* created by Pringsheim (1858) consists of soil inhabiting fungi which exist both as pathogens and saprophytes (Garrett, 1956). *Pythium* spp. cause failure of germination of seeds as well as post emergence damping off of seedlings and root rot in adult plants.

Although there is little doubt about the widespread occurrence *Pythium* spp. in soil there are very few records of their direct isolation from this substratum. A scrutiny of Gilman's (1957) "Manual of soil fungi" indicates that only 17 out of the 73 known species of *Pythium* have been directly recorded from soil. This is in contrast to such groups as Mucorales where out of 250 species as many as 129 have been directly recorded from soil. In the former case each species has been recorded just once or twice.

The primary reason for this disparity is no doubt the selectivity of the dilution plate technique (Waksman, 1922; Brierley *et al*, 1927) most commonly used by most soil Mycologists for the isolation of soil fungi. This selectivity has been discussed by Chester's (1956). Johnson *et al* (1959) also comment on the peculiarity of the dilution plate technique and say that this technique favours fungi that sporulate abundantly in soil. It is the common experience of most investigators in soil mycology, including the authors that *Pythium* spp. occur most infrequently on dilution plates.

Realising the limitations of the dilution plate technique many investigators have attempted to modify it so as to make it less selective. Because of the loss of larger soil particles in the pipette transfers used in the dilution plates, Menzies (1957) devised a dipper for pouring serial dilutions. Whether by this method *Pythium* species were isolated from soils is not clear from Menzies' note.

Some modifications of Menzies' technique have been suggested by Paharia and Kommendahl (1954) who poured the soil dilutions two or three days after the agar plates were poured. They also used rose bengal and streptomycin to supress bacterial growth. They reported that *Pythium* species appeared in plates where rose bengal or streptomycin had been added but it is not clear how frequently.

By using the soil plate method Warcup (1950, 1951) was able to isolate two species of *Pythium* but the frequency of their appearance was not great. Thornton's (1922) screened immersion plate technique did not yield any *Pythium* species from soil.

The immersion tube devised by Chester's (1940, 1948) has been found useful for the study of actively growing soil fungi. Four species of *Pythium* were isolated by this technique by Chesters (1948).

Meredith (1940) isolated six species of *Pythium* from soil by planting of small portions of soil on nutrient free agar and has made some preliminary observations on the frequency of occurrence of these species.

The slide trap technique of La Touche (1948) and its later modification of Sewell (1956) did not yield any *Pythium* spp. from soil.

Angell (1954) reported a technique by which estimation of the distribution and number's of *Pythium* spp. was possible. The method consisted of fractionation of the soil and separation of colloidal particles. The bacteria were predominently present in the colloidal fraction and the *Pythia* in the coarser fractions.

Nour (1956) isolated several fungi including two *Pythium* species from Sudan soils by using Waksman's dilution method, Warcup's soil plate method and Chesters' immersion tube technique.

The technique of Campbell (1951) and Tsao (1960) are highly selective and were designed for isolation of pathogenic species of *Phytophthora*. The technique of Barton (1958) was also selective and yielded pathogenic species of *Pythium* from soil.

The techniques usually adopted for the isolation of *Pythium* species from soil is the baiting technique. Butler (1907) was the first to use this technique extensively for the study of *Pythium* and Chytridiaceae. Butler was able to isolate 18 species of *Pythium* and 11 species of Chytrids by this method. The baiting technique has been extensively used by many later workers like Sparrow (1943), Couch (1939), Karling (1945) and Nicholls (1956) for the isolation of Phycomyceteous fungi from soil and water. Most of these authors however, studied other groups of Phycomycetes than *Pythium*. However, it is surprising that the baiting technique has not been used to any extent for the study of ecology of *Pythium* spp. in agricultural and other soils.

The agar block technique described by Rangaswamy (1958) was reported to be good for the isolation of soil fungi especially Pythiaceous fungi. This technique being very recent has not been extensively tried out till now.

In the present studies a comparison of three techniques viz., the dilution plate, the baiting technique and the agar block technique was attempted to assess their suitability to isolate Phycomycetes from four soils. The dilution plate technique was abandoned after a few preliminary trials as it failed to yield any *Pythium* spp.

The baiting technique and the agar block technique were extensively compared for their ability to isolate *Pythium* spp. from the four agricultural soils over a period of 12 months. In the baiting technique three baits viz., cockroach wing, ant and cellophane pieces were used. A glance at table 1 and 4 would indicate that both the baiting technique and the agar block technique yielded large numbers of *Pythium* spp. The baiting technique in addition, also yielded a large number of species of Chytridiales. It was observed that the kind of bait used strongly influence the numbers of fungi isolated. For example the cockroach wing bait yielded significantly larger numbers of *Pythium* spp. than either the ant or the cellophane bait.

The baits had a greater affinity for Chytridiales than for *Pythium* spp. It was also clear from the results htat the kind of bait used strongly influenced the number of isolates of *Pythium* spp., cockroach wing bait giving the largest number of isolations and cellophane pieces the least. Ant bait was on a par with cockroach wing bait in most cases. It was however, interesting to note that the comparative abundance of *Pythium* species in the four soils remained the same in isolations made by all the baits in all the months.

In the isolation of *Pythium* species it is observed that cockroach wing and ant bait rarely differed in their efficacy but as regards the isolation of Chytridiales cockroach wing bait was largely the most efficient bait.

The bait also indicated the differences in abundance of occurrence of *Pythium* and Chytridiales between the different months of the year. The comparative abundance remained the same whatever be the baits used. All the baits indicated that *Pythium* spp. and Chytridiales occur in greater abundance during July to December than during the rest of the months

In the present studies it was observed that streptomycin at 1000 p.p.m. was useful and effective in inhibiting the growth of bacteria. Streptomycin sulphate as a bactericide gave significantly greater number of isolates of *Pythium* spp. and Chytridiales. Streptomycin sulphate was more efficient as a bactericide than lactic acid. Martin (1950) found the use of the peptone dextrose agar containing 1:30,000 rose bengal and 30 μg of streptomycin to give useful results in the isolation of fungi.

Agar block technique: The agar block technique did not yield any Chytridiales from soil, but was found to be very satisfactory for the isolation of *Pythium* spp. When the agar block and the baiting technique were compared it was observed that the agar block yielded more number of *Pythium* than *any one* of the baits used in the baiting technique. It was also observed that a greater variety of species of *Pythium* was obtained by the agar block technique. Both the agar block and the baiting technique showed the same comparative abundance of *Pythium* spp. in the four soils studied. The agar block technique also showed that there was a greater abundance of *Pythium* spp. in the four soils during July to December. It can thus be seen that the two techniques corroborate each other. The agar block technique was however, chosen for further studies because of its merits already pointed out and also because this technique enabled the isolation of fungi from soils *in situ* without the least possible disturbance of the soil profiles.

The agar block technique can be compared to the screened immersion plate technique of Thornton (1952) and the immersion tube of Chesters (1940, 1948). By the screened immersion plate technique no *Pythium* spp. has been isolated, however, while by the immersion tube technique it was possible to isolate a few. The greater ability of the agar block technique to isolate *Pythium* seems to be because of the wide area of colonisable substratum exposed to the fungi in soil. There is moreover intimate contact of the agar medium with the soil particles and diffusion of moisture and nutrients into a small area of soil surrounding the agar block. The suitability of the agar block technique for the isolation of *Pythia* may, perhaps also be attributed to the fact that most of the *Pythia* belong to the category of "sugar fungi" which are primary colonisers of substrata rich in easily assimilable carbohydrates. A recent study of Barton (1958) has shown that the activity of

Pythium mamillatum in soil was greatly increased by the addition of maize meal.

Certain limitations of the agar block technique may also be pointed out here. By this technique it is not possible to determine absolute numbers of *Pythium* spp. for a given weight of soil. It is possible only to assess their comparative abundance of occurrence in different soils. This disadvantage is however, shared by other techniques like immersion tube technique and straw burial and root burial technique of Sadasivan (1939) and Subramanian (1946). Another limitation of this technique is that the media used may exercise a certain amount of selectivity towards particular species of *Pythium* in soil. But this disadvantage may be overcome by using different media.

Factors affecting distribution and abundance of fungi in soils: It has been reported by various workers that the distribution and abundance of fungi vary from soil to soil. Waksman (1916) has emphasized the fact that fungi occur in large numbers in cultivated as well as in uncultivated soils. Several fungi have been isolated, described and their numbers estimated from time to time from cultivated soils (Chand, 1937; Chaudhuri and Sachar, 1934; Jensen, 1931; Singh, 1937) from forest soils (Ellis, 1940; Paine, 1927) from soils with high organic matter content, (Verona, 1934) and from cultivated as compared with natural soils (Bisby *et al*, 1933; Dixon, 1928). Chesters (1949) found that *Pythium* spp. were specifically prolific in cultivated soils under vegetable crops. Barton (1958) was of opinion that *Pythium* spp. were absent or at the most sparsely distributed in non-cultivated soils. Remy (1949) concluded that *Pythium* spp. were abundant in cultivated soils and frequent in forest soils as well as those of a dry sandy nature. Warcup (1952) showed the predominance of these parasites in nursery soils but they were absent in soils from virgin forest areas.

In the present investigation, the distribution of *Pythium* in the four agricultural soil types of the cultivated areas attached to the Agricultural College and Research Institute, Coimbatore was studied. These soil types are subjected to different agronomic practices which conform to the agricultural practices of Madras State in general and Coimbatore in particular. The investigations were spread over a period of twelve months commencing from April 1957 to March 1958 coinciding with the cropping seasons of the soil types.

The number of *Pythium* isolated from the four soils differed from month to month in all the soils (table 4 and Text Fig. 1), maximum number of isolates being obtained during August while the period from January to March recorded the lowest number of isolates. The trend of distribution was observed to be similar in all the four soils studied. This indicates that different factors contribute to the distribution of fungi which are worthy of consideration. Various physical, chemical and biological factors bring about these differences but these have not, however, been adequately understood, as continuous studies of a single soil or a group of soils have been rare.

Summary

The objective of the present investigation was to study the distribution and behaviour of Phycomycetous fungi in four agricultural soil types with special reference to Pythiaceae. Four soil types viz., dry land (rainfed), garden land, wet land and semi-wet land were chosen for the study. These soils were cropped to millets, vegetables, rice and banana respectively.

Two techniques, viz., the baiting technique and the "agar block" technique were employed for the isolation of *Pythium* species from four different soil types. In the baiting technique cockroach wing, ant and cellophane were used as baits. Both the techniques, viz., the "baiting" and the "agar block" were found suitable for the isolation of *Pythium*, but the agar block technique was more efficacious, yielding more numbers of *Pythium* and greater variety of species. Among the baits used cockroach wing bait was the best for the isolation of *Pythia* followed by ant; cellophane was unsuitable. For the isolation of Chytridiales cockroach wing bait was the best bait followed by cellophane. The agar block technique was more advantageous probably because a wide area of colonisable substratum is exposed to fungi in the soil. But the limitation of this technique was that while it can be used for assessing comparative numbers of *Pythia* in different soils or in the same soil at different periods it cannot be used for estimating the absolute numbers.

The number of *Pythium* isolates varied from month to month in all the four soils, the maximum number being isolated in the month of August. This trend of distribution was observed to be similar in all the four soils.

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